Magna Scientia Advanced Research and Reviews

eISSN: 2582-9394

Cross Ref DOI: 10.30574/msarr

Journal homepage: https://magnascientiapub.com/journals/msarr/

(RESEARCH ARTICLE)

Pregnancy Rates in Day 3, 4, and 5 Embryo Transfers in IVF Cycles: A Comparative Study

Firnanda Bagoes Septiawan Putra [1], Zakiyatul Faizah [2,*], Sri Ratna Dwiningsih [3] and Ninik Darsini [2]

[1] *Medical Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[2] *Department of Medical Biology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[3] *Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia.*

Magna Scientia Advanced Research and Reviews, 2025, 15(02), 010-017

Publication history: Received on 24 September 2025; revised on 03 November 2025; accepted on 06 November 2025

Article DOI: https://doi.org/10.30574/msarr.2025.15.2.0131

Abstract

Background: Infertility affects about 1 in 6 people worldwide, including 10–15% of reproductive-age couples in Indonesia. In vitro fertilization (IVF) is a key technology to overcome infertility, with success influenced by factors such as patient age and embryo transfer timing. This study aims to compare pregnancy rates based on patient age and embryo transfer days (3, 4, and 5) to support more effective clinical decision-making in IVF programs.

Objectives: Comparing and analyzing pregnancy rates in IVF program patients based on patient age and embryo transfer on days 3, 4, and 5.

Materials and Methods: A cross-sectional analytical study used medical records of 460 IVF patients at Tiara IVF Clinic, Dr. Soetomo General Hospital Surabaya (2018–2023). Biochemical and clinical pregnancy rates were analyzed according to patient age and embryo transfer day (day 3, 4, 5) using Chi-square tests.

Results: The highest biochemical and clinical pregnancy rates were observed in the 26-30 age group and embryo transfer on day 4. The *Chi Square* test for the age variable showed a significant difference with p=0.009 for biochemical pregnancy and p<0.001 for clinical pregnancy. The *Chi Square* test for the embryo transfer day variable also showed a significant difference with p=0.007 for biochemical pregnancy and p=0.001 for clinical pregnancy.

Conclusion: There were significant differences in biochemical and clinical pregnancy rates based on patient age and embryo transfer day. Limited sample size and other unobserved factors remain important limitations in this study.

Keywords: Maternal age; Day 3; Day 4; Day 5; Embryo transfer; Chemical pregnancy; Clinical pregnancy; IVF

1. Introduction

Human reproduction is fundamental to the continuity and sustainability of human existence. However, reproductive function does not always operate optimally, as various biological, environmental, and lifestyle factors can interfere with fertility. Approximately one in six individuals globally experiences infertility, making it a significant public health issue that affects both developed and developing nations alike [1]. In Indonesia, the prevalence of infertility is estimated to reach 10–15%, affecting around 4–6 million couples of reproductive age [2]. This condition not only influences individual and family well-being but may also have broader demographic implications, potentially reducing population growth rates and impacting the country's opportunity to achieve its demographic dividend by 2035.

* Corresponding author: Zakiyatul Faizah

Over the past few decades, advancements in reproductive medicine have led to the development of various assisted reproductive technologies (ARTs) to address infertility. Among these, in vitro fertilization (IVF) has emerged as one of the most effective and widely applied methods. Since the birth of the first IVF baby in 1978, this technique has evolved significantly, providing new hope for couples struggling with infertility [3]. IVF involves the fertilization of oocytes outside the human body and subsequent transfer of the resulting embryo into the uterus. The use of IVF has expanded globally, not only for couples with infertility but also for women who delay childbearing due to social, economic, or professional considerations, such as pursuing higher education and career advancement [4].

The success of IVF treatment is determined by multiple factors, including maternal age, ovarian reserve, sperm quality, fertilization rate, embryo grading, and endometrial receptivity [5]. Advances in embryo culture media and laboratory techniques have further enhanced embryo viability and implantation potential. Additionally, the type of embryo used—fresh or frozen—can affect pregnancy outcomes, with several studies reporting slightly higher success rates with frozen embryo transfers due to improved endometrial synchronization [6], [7].

One crucial step in IVF is the timing of embryo transfer, which is typically performed on day 3 (cleavage stage) or day 5 (blastocyst stage) after fertilization. Each stage offers distinct clinical advantages and challenges. Day 3 transfers allow for earlier embryo placement and may be preferred when embryo development in vitro is uncertain. In contrast, day 5 transfers enable better selection of viable embryos with higher implantation potential but may lead to the loss of embryos that fail to reach the blastocyst stage. Previous studies have suggested that day 5 transfers yield higher pregnancy rates while requiring fewer embryos to achieve successful implantation [6], [7]. However, other studies, including those by Rifasky et al. and Garbhini et al. have reported no statistically significant difference in pregnancy outcomes between day 3 and day 5 transfers [6], [7]

In certain clinical situations, some practitioners opt for day 4 embryo transfers as a compromise between the cleavage and blastocyst stages. This approach can be beneficial for patients with limited embryo numbers or suboptimal development, helping to minimize embryo loss before reaching the blastocyst stage [8]. Despite this, the body of comparative data regarding pregnancy outcomes among day 3, 4, and 5 embryo transfers remains limited.

Given the ongoing debate and the paucity of comprehensive comparative evidence, this study aims to analyze and compare pregnancy rates based on embryo transfer timing—specifically days 3, 4, and 5—while also considering maternal age as a key influencing factor. The findings are expected to provide valuable insights for clinicians and embryologists in optimizing embryo transfer strategies and improving clinical decision-making to enhance IVF success rates.

2. Material and methods

This study employed an analytic observational design with cross-sectional approach, in which data on the studied variables were collected at a single point in time from the target population. The study population consisted of all patients who underwent in vitro fertilization (IVF) procedures at Tiara IVF Clinic, Graha Amerta, Dr. Soetomo General Hospital, Surabaya, during the period 2018–2023. A total sampling technique was applied, meaning that all cases within the population that met the inclusion criteria were included in the study. This study has been approved by the Research Ethics Committee at Dr. Soetomo Hospital Surabaya (No. 1334/KEPK/VI/2025). The patient's name identity is not written in the study so that the identity is maintained, the confidentiality of patient data will be maintained by the author, and all information obtained is only used for research purposes.

3. Results and discussion

Based on patient data from Tiara IVF Clinic, Graha Amerta, Dr. Soetomo General Hospital, Surabaya, collected between 2018 and 2023, a total of 671 cases were identified. After applying the predefined inclusion and exclusion criteria, 460 cases met the eligibility requirements and were included in the final analysis. The samples in this study were taken from various age categories to see how the pregnancy outcomes obtained. All samples were considered optimal, with endometrial thickness greater than or equal to 11 mm and all embryos rated as good, i.e., at least grade 8C on day 3, grade M on day 4, and grade B on day 5.

Table 1 Data on age groups with biochemical pregnancy rates

			Biochemical Pregnancy		**Total**	***p-value***
			Positive	Negative		
Age Group	20–25	N	5	10	15	0,009
		(%)	(33.3%)	(66.7%)	(100%)	
	26–30	N	63	51	114	
		(%)	(55.3%)	(44.7%)	(100%)	
	31–35	N	94	101	195	
		(%)	(48.2%)	(51.8%)	(100%)	
	36–40	N	42	73	115	
		(%)	(36.5%)	(63.5%)	(100%)	
	>40	N	5	16	21	
		(%)	(23.8%)	(76.2%)	(100%)	
Total		N	209	251	460	
		(%)	(45,4%)	(54.6%)	(100%)	

Table 2 Data on age groups with clinical pregnancy rates

			Clinical Pregnancy		**Total**	***p-value***
			Positive	**Negative**		
Age Group	20–25	N	3	12	15	<0,001
		(%)	(20%)	(80%)	(100%)	
	26–30	N	58	56	114	
		(%)	(50.9%)	(49.1%)	(100%)	
	31–35	N	84	111	195	
		(%)	(43.1%)	(56.9%)	(100%)	
	36–40	N	39	76	115	
		(%)	(33.9%)	(66.1%)	(100%)	
	>40	N	2	19	21	
		(%)	(9.5%)	(90.5%)	(100%)	
Total		N	186	274	460	
		(%)	(40.4%)	(59.6%)	(100%)	

Table 1 and Table 2 demonstrate statistically significant differences ($p<0.05$) in both biochemical and clinical pregnancy outcomes across maternal age categories. The highest pregnancy rates were observed among women aged 26–30 years, whereas the lowest rates were found in those aged over 40 years, for both biochemical and clinical pregnancy indicators. These results underscore the critical role of maternal age as a determinant of IVF success, reaffirming that reproductive potential declines substantially with advancing age. Similar findings have been reported by Wang et al. and Sun et al., who observed that increasing maternal age was significantly associated with reduced biochemical and clinical pregnancy rates, with a marked decline noted after the age of 40 [9], [10]

However, the present findings partially contrast with those of Bagheri et al. and Meng et al., who reported no statistically significant differences among age groups, despite observing a comparable downward trend in pregnancy outcomes

among older patients [11], [12]. These inconsistencies across studies may be attributed to variations in study design, sample size, ovarian stimulation protocols, and embryo selection criteria, all of which could influence statistical outcomes.

The biological basis of age-related fertility decline has been widely discussed in reproductive medicine. Diminished ovarian reserve and impaired folliculogenesis are among the primary mechanisms explaining reduced reproductive outcomes in older women. As age increases, the number and quality of oocytes decline due to accumulated oxidative stress, mitochondrial dysfunction, and chromosomal instability, leading to higher rates of aneuploidy and embryonic developmental arrest [13]. Consequently, the likelihood of achieving a viable embryo that can implant and sustain pregnancy decreases significantly.

Beyond oocyte quantity and quality, recent studies have also highlighted the importance of embryo competence and molecular factors in determining implantation success. Embryo quality is influenced not only by morphological assessment but also by gene expression profiles and epigenetic modifications, which regulate cell differentiation and development during early embryogenesis [14]. Epigenetic alterations, such as DNA methylation and histone modification, are increasingly recognized as mediators linking maternal age to embryo developmental potential.

Moreover, endometrial factors play an equally important role in reproductive outcomes. Endometrial receptivity tends to decline with age due to epigenetic dysregulation, altered expression of implantation-related genes, and changes in hormonal responsiveness [15]. These molecular and structural alterations can result in a suboptimal uterine environment, reducing the likelihood of successful implantation even when morphologically normal embryos are transferred.

Taken together, these findings reinforce that maternal age exerts a multifactorial influence on IVF outcomes through its impact on both gamete and endometrial quality. The observed age-dependent decline in biochemical and clinical pregnancy rates emphasizes the need for early fertility assessment, patient counseling, and potentially tailored clinical strategies—such as preimplantation genetic testing (PGT), embryo freezing at younger ages, or oocyte preservation—to optimize success rates in assisted reproductive programs.

Table 3 Data on day 3, 4, and 5 embryo transfers with biochemical pregnancy rates

			Biochemical Pregnancy		**Total**	***p-value***
			Positive	**Positive**		
Day of Embryo Transfer	Day 3	N	88	141	229	0,007
		(%)	(38.4%)	(61.6%)	(100%)	
	Day 4	N	24	17	41	
		(%)	(58.5%)	(41.5%)	(100%)	
	Day 5	N	97	93	190	
		(%)	(51.1%)	(48.9%)	(100%)	
Total		N	209	253	462	
		(%)	(45.2%)	(54.8%)	(100%)	

Table 4 Data on day 3, 4, and 5 embryo transfers with clinical pregnancy rates

			Clinical Pregnancy		**Total**	***p-value***
			Positive	**Positive**		
Day of Embryo Transfer	Day 3	N	74	155	229	0,001
		(%)	(32.3%)	(67.7%)	(100%)	
	Day 4	N	23	18	41	
		(%)	(56.1%)	(43.9%)	(100%)	

	Day 5	N	89	101	190	
		(%)	(46.8%)	(53.2%)	(100%)	
Total		N	186	276	462	
		(%)	(40.3%)	(59.7%)	(100%)	

Table 3 and Table 4 demonstrate statistically significant differences ($p<0.05$) in both biochemical and clinical pregnancy outcomes among day-3, day-4, and day-5 embryo transfer groups. The highest biochemical and clinical pregnancy rates were recorded in the day-4 transfer group, followed by day-5 and day-3 transfers, respectively. These findings suggest that the timing of embryo transfer plays a crucial role in determining IVF success rates. Interestingly, the superior outcomes observed in day-4 transfers differ from those reported by Borzouie et al. and Kaartinen et al., who found no statistically significant differences between day-3 and day-4 transfers, despite noting similar upward trends in pregnancy rates with later-stage embryos [16], [17]. Similarly, Li et al. and Lee et al. reported no significant variations in pregnancy outcomes between day-4 and day-5 transfers, suggesting that clinical success may not be determined solely by the developmental stage of the embryo but also by synchronization with the endometrial environment [18], [19].

The improved outcomes associated with day-4 transfers may be explained by optimal alignment between embryo developmental stage and endometrial receptivity. On day 4 post-fertilization, the embryo typically reaches the morula stage, during which the maternal-to-embryonic genome transition (MZT) occurs, enabling the embryo to begin autonomous gene expression and metabolic regulation. At this point, the embryo is naturally expected to enter the uterine cavity in vivo, typically around 3–4 days after fertilization. Performing embryo transfer at this time may therefore create a more physiologically synchronized interaction between the embryo and endometrium, enhancing the likelihood of successful implantation. Furthermore, several studies have suggested that uterine contractility decreases by day 4, potentially minimizing mechanical disruption and improving the probability of embryo adherence to the endometrial lining [20], [21].

In contrast, the lower pregnancy outcomes observed in day-3 transfers may be attributed to the earlier stage of embryonic development, during which the embryo remains in the *cleavage* phase and has yet to undergo compaction. At this point, embryonic genome activation is incomplete, and the embryo may be less capable of establishing the biochemical communication required for implantation. Meanwhile, the relatively high success rates seen in day-5 transfers have often been associated with enhanced embryo selection at the *blastocyst* stage, as only the most viable embryos typically progress this far in culture. This stage allows embryologists to assess morphological and developmental quality more accurately, selecting embryos with the greatest implantation potential [22]. However, extending culture to day 5 also carries risks, such as embryo degeneration or developmental arrest before reaching the blastocyst phase, which can reduce the total number of transferable embryos—particularly in patients with low ovarian response.

Taken together, these results suggest that day-4 embryo transfer may offer a favorable balance between the biological advantages of extended culture and the practical considerations of embryo survival and endometrial readiness. This intermediate timing might optimize the embryo–endometrium dialogue necessary for implantation, especially in patients where blastocyst development rates are uncertain. The findings highlight the importance of individualized embryo transfer strategies based on patient characteristics, embryo quality, and laboratory conditions to maximize pregnancy outcomes in IVF programs.

An interesting finding from this study is the observed decrease in clinical pregnancy rates compared with biochemical pregnancy rates. This pattern indicates that not all biochemical pregnancies successfully progress to the clinical stage, reflecting the occurrence of early pregnancy losses. Biochemical pregnancy is typically defined by the detection of elevated serum β-hCG levels following embryo transfer, without subsequent visualization of a gestational sac on ultrasonography. In contrast, clinical pregnancy requires sonographic confirmation of an intrauterine gestational sac, usually around 4–6 weeks after transfer. The discrepancy between biochemical and clinical pregnancy rates thus highlights that a subset of conceptions detected at the biochemical level may fail to establish a sustainable implantation.

This phenomenon is frequently attributed to *transient implantation*—a situation in which the embryo initially attaches to the endometrium and begins to secrete hCG but does not continue to develop into a viable pregnancy. Such early pregnancy loss may occur due to chromosomal or genetic abnormalities within the embryo that prevent further cell division or differentiation, leading to embryonic arrest and subsequent resorption [23], [24]. Studies in reproductive

genetics suggest that up to 50–70% of early pregnancy losses in assisted reproductive technologies (ART) are associated with aneuploidy, particularly in embryos derived from women of advanced maternal age.

Beyond genetic causes, endometrial factors also play a crucial role in determining whether a biochemical pregnancy progresses to a clinical one. Inadequate endometrial receptivity, altered expression of implantation-related genes, and epigenetic modifications can all disrupt the complex molecular dialogue required for stable implantation. Endometrial dysfunction may lead to impaired trophoblast invasion and suboptimal uterine blood flow, thereby preventing the establishment of a viable gestation [24]. Hormonal imbalances—particularly insufficient luteal phase support or inadequate progesterone response—may also contribute to failed progression from biochemical to clinical pregnancy by compromising the endometrial environment needed to sustain early embryonic development.

Advanced maternal age is another critical factor that may explain this trend. Older women are known to have a higher incidence of oocyte and embryo chromosomal abnormalities, mitochondrial dysfunction, and diminished cytoplasmic integrity, all of which compromise embryo viability. Moreover, age-related alterations in endometrial receptivity, including reduced responsiveness to estrogen and progesterone, decreased expression of adhesion molecules, and increased oxidative stress, further reduce the likelihood of successful implantation and maintenance of early pregnancy [25]. Consequently, these combined factors result in a higher proportion of biochemical pregnancies that fail to progress into clinically confirmed pregnancies among older patients.

Overall, the observed decline in clinical pregnancy rates compared to biochemical rates underscores the multifactorial nature of early pregnancy failure in IVF cycles. This emphasizes the importance of comprehensive approaches aimed at improving both embryo and endometrial quality—such as preimplantation genetic testing (PGT) to select euploid embryos, optimization of endometrial preparation protocols, and individualized luteal support regimens. By addressing these biological and clinical variables, the gap between biochemical and clinical pregnancy rates may be reduced, ultimately enhancing overall IVF success and live birth outcomes.

4. Conclusion

The highest biochemical and clinical pregnancy rates were observed in the 26–30-year age group and in day-4 embryo transfers. Statistical analysis demonstrated significant differences in pregnancy outcomes based on both maternal age and embryo transfer timing (day 3, 4, and 5), for biochemical and clinical pregnancies. Biochemical pregnancy rates were consistently higher than clinical pregnancy rates across age groups and transfer days. These findings indicate that maternal age and embryo transfer timing are important factors influencing implantation success and pregnancy outcomes IVF programs.

Compliance with ethical standards

Acknowledgments

The authors would like to express their deepest gratitude to the staff of Dr. Soetomo General Hospital, Surabaya, as well as to the members of the Faculty of Medicine, Airlangga University, Surabaya, for their dedicated assistance and meaningful contributions to the completion of this study.

Disclosure of conflict of interest

The authors declared there's no conflict of interest.

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