

Impact of Oocyte Denudation Timing on Fertilization Rates and Blastocyst Development: A Systematic Literature Review

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ABSTRACT

Background: There is ongoing debate regarding the timing of denudation during In-vitro Fertilization (IVF) or Intra Cytoplasmic Sperm Injection (ICSI) procedures because of its impact on oocyte quality and treatment outcomes. A process that involves both enzymatic treatment and mechanical stripping can influence oocyte quality and maturity. For ICSI, denudation helps assess oocyte maturity and quality, while in IVF, cumulus cells are typically removed after fertilization to evaluate its success. Yet, the optimal timing for denudation is still not well-defined, necessitating further studies to understand its impact on assisted reproductive technology (ART) results.

Purpose: This review aims to assess how the timing of denudation affects fertilization rates and the formation of blastocysts in ART procedures.

Methods: We conducted a systematic review of literature published between 2003 and 2024. A total of 32 articles were reviewed, and 12 studies that met the inclusion criteria were included. These consisted of original published research reporting on fertilization rates, pregnancy rates, embryo quality, and live birth rates. Animal studies were excluded from this review.

Results: This study shows that with denudation exceeding more than 5 hours, fertilization rates decline significantly, reaching as low as 27.3% at the 8-hour mark. The objective for the oocyte denudation timing interval from oocyte retrieval to ICSI is less than 5 hours to enhance the chances of fertilization. However, limited data on blastocyst formation are available; therefore, further studies are required to better understand the overall impacts on fertilization, embryo development, and clinical pregnancy rates.

Conclusion: Although the best denudation timing of oocytes is still unclear in ART, a pre-incubation of cumulus oocyte complex (COCs) for one to four hours post ovum pickup may be beneficial. An interval of more than four hours in COC denudation might be harmful. It is necessary to conduct further studies with upgraded protocols in order to create evidence-based guidelines to improve oocyte maturation, fertilization, and blastocyst development.



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1. Introduction

The timing between oocyte extraction and fertilization is a key factor, as denudation plays a crucial role regarding oocyte quality, fertilization viability, eventually, embryonic development. Oocyte denudation enables embryologists to evaluate oocyte maturity and quality, which are necessary for determining whether the oocyte is ready for fertilization. In addition, cumulus cells need to be eliminated to provide a clear view of the oocyte for ICSI, which allows the injection of a single sperm into it with the utmost precision.

Insemination post oocyte retrieval is a controversial aspect considered by ART practitioners (Ho *et al.*, 2023). Oocyte equilibration or pre-incubation in a 6% CO₂ environment prior to the IVF or ICSI procedure has been suggested to ensure

that oocyte maturation has been completed. Previous studies reported that pre-incubation has no remarkable effect on embryonic growth. Additional research is required to determine the impact of denudation timing on oocyte competence, fertilization, and embryo development. Particularly in IVF and ICSI procedures, the timing and process of denudation can have a significant impact on ART outcomes.

The method of oocyte denudation timing varies depending on the ART procedures. Oocyte denudation is essential before the ICSI procedure because it provides a clear view for oocyte assessment, proper grading of the maturation stages such as metaphase I and II (MI & MII), and better oocyte holding with good control in the holding pipette, making it easier to orient the oocyte for

injection at the correct position, either at the 12 o'clock or 6 o'clock polar body position. However, in conventional in-vitro fertilization (c-IVF) procedures, oocyte denudation is roughly performed 8 to 16 hours post insemination for evaluation of fertilization status and zygote quality.

In c-IVF procedures, if oocyte denudation is performed after a prolonged denudation timing interval, it may have negative effects on fertilization and embryonic development, indicating the need to refine denudation timing to improve ART outcomes. Although this literature review paper provides varied information about the effect of late denudation timing on fertilization and blastocyst development, some studies show no loss or insignificant loss of zygote formation and blastocyst development in late denudation post oocyte retrieval before ICSI or in c-IVF. Some authors suggest that late denudation timing may have a possible negative impact on fertilization and embryo development. These conflicting results emphasize the imperative need to improve denudation timing to enhance ART success, and more research is needed to clarify the effect of denudation timing on fertilization, embryo development, and live birth rate.

2. Literature Review

We conducted a review of the literature on the timing of denudation and its association with fertilization and blastocyst formation. Longer incubation of cumulus oocyte complexes (COCs) after oocyte pickup has been shown to improve oocyte maturation and fertilization, whereas incubation beyond 4 hours may reduce the clinical pregnancy rate. However, data on the effect of denudation timing on blastocyst development are lacking and require further study.

COCs equilibration 2–4 hours post ovum pick-up is sufficient for oocyte maturation, and denudation should be performed within this time duration to minimize the negative

3. Results

3.1. Effect of Denudation Time on Fertilization and Blastocyst Formation Rates

Table 1: Fertilization Rates According to Denudation Timing (0–2.5 hours)

Sr. No.	Author(s)	Sample Size	Denudation Time	Procedure	Fertilization Rate (%)	Year	References
1	Fatma Aletebi <i>et al.</i>	132 oocytes	0 hours	ICSI	70.7	2011	(Aletebi <i>et al.</i> , 2011)
2	Zehra Beyza Düzgün <i>et al.</i>	376 oocytes	0 hours	ICSI	55.6	2022	(Düzgün <i>et al.</i> , 2022)
3	Satoshi Mizuno <i>et al.</i>	269 oocytes	0 hours	ICSI	88.4 ± 10.20	2018	(Mizuno <i>et al.</i> , 2018)

impact on fertilization, embryo development, blastocyst formation, and clinical pregnancy rates. More studies are needed to establish the relationship between the timing of denudation and ART (c-IVF/ICSI) outcomes, especially blastocyst development and clinical pregnancy outcomes.

2.1. Review Objectives

This study shows the optimal timing for oocyte denudation in order to increase the success rate of IVF/ICSI and to assess whether early denudation can improve fertilization rates.

2.2. Review Questions

1. Does the timing of denudation influence fertilization rates and blastocyst formation in IVF/ICSI?
2. Is there an optimal denudation time for maximizing embryo quality and blastocyst formation?
3. How does delayed denudation affect IVF/ICSI outcomes, particularly fertilization and embryo quality, as well as pregnancy rates?
4. What is the ideal timing for denudation in IVF/ICSI cycles to achieve the highest fertilization rates?

2.3. Review Methodology

A systematic literature review was carried out to identify studies published between 2003 and 2024. A total of 32 articles were identified, of which 12 met the inclusion criteria and were included in the final analysis.

- **Inclusion criteria:** Articles should represent original research with full text available, focusing on outcomes such as fertilization rates, pregnancy rates, embryo quality, and live birth rates.
- **Exclusion criteria:** Animal studies and studies lacking comprehensive data on primary outcomes were excluded.

4	Fatma Aletebi <i>et al.</i>	72 oocytes	1 hour	ICSI	69.6	2011	(Aletebi <i>et al.</i> , 2011)
5	Jingbo Chen <i>et al.</i>	246 oocytes	1 hour	ICSI	25	2023	(Chen <i>et al.</i> , 2023)
6	Jason Yen-Ping Ho <i>et al.</i>	96 oocytes	2 hours	ICSI	56.3	2003	(Ho <i>et al.</i> , 2003)
7	Fatma Aletebi <i>et al.</i>	105 oocytes	<1 hour	ICSI	74.8	2011	(Aletebi <i>et al.</i> , 2011)
8	Osama Naji <i>et al.</i>	1118 oocytes	<2 hours	ICSI	55	2018	(Naji <i>et al.</i> , 2018)
9	Zehra Beyza Düzgün <i>et al.</i>	416 oocytes	2 hours	ICSI	48.3	2022	(Düzgün <i>et al.</i> , 2022)
10	Jingbo Chen <i>et al.</i>	246 oocytes	1–2 hours	ICSI	23.68	2023	(Chen <i>et al.</i> , 2023)
11	Satoshi Mizuno <i>et al.</i>	398 oocytes	2 hours	ICSI	88.0 ± 11.2	2018	(Mizuno <i>et al.</i> , 2018)
12	Jason Yen-Ping Ho <i>et al.</i>	256 oocytes	2.5 hours	ICSI	54.7	2003	(Ho <i>et al.</i> , 2003)

Table 1 shows the effect of timing of oocyte denudation on subsequent ICSI fertilization rates. Denudation is the removal of surrounding cumulus cells. Results are highly variable across studies, making it impossible to determine a clinically relevant optimal interval. Studies using immediate denudation (0 hours) reported fertilization rates from a low of 55.60% (Düzgün *et al.*, 2022) to a high of 88.4% (Mizuno *et al.*, 2018). Delaying denudation to 1 hour produced results with extremes from a low of 25.00% (Chen *et al.*, 2023) to a high of 69.60% (Aletebi *et al.*, 2011). Further extending the delay to 2 hours showed rates

between these extremes of 48.30% (Düzgün *et al.*, 2022) and an upper extreme value of 88.0% (Mizuno *et al.*, 2018), with a slight further delay to an intermediate time point of 2.5 hours yielding a lower figure of 54.70% (Ho *et al.*, 2003). Such large heterogeneity, even among studies using similar time points, strongly underlines the possible role that other confounding factors might play in influencing outcomes, such as oocyte quality, patient demographics, or specific laboratory protocols; thus, indicating that although denudation timing is one critical variable, it may not be the only determinant of ICSI success.

Table 2: Fertilization Rates According to Denudation Timing (2– 5 hours)

Sr. No.	Author(s)	Sample Size	Denudation Time	Procedure	Fertilization Rate (%)	Year	References
1	Patrizia Falcone <i>et al.</i>	1228 oocytes	2–3 hours	ICSI	71.1	2008	(Falcone <i>et al.</i> , 2008)
2	Osama Naji <i>et al.</i>	933 oocytes	2–3 hours	ICSI	55	2018	(Naji <i>et al.</i> , 2018)
3	M. Carvalho <i>et al.</i>	1378 cycles	<3 hours	ICSI	62.00 ± 22.70	2020	(Carvalho <i>et al.</i> , 2020)
4	Jingbo Chen <i>et al.</i>	246 oocytes	2–3 hours	ICSI	19.9	2023	(Chen <i>et al.</i> , 2023)
5	Catherine Patrat <i>et al.</i>	903 oocytes	3 hours	ICSI	65.8	2012	(Patrat <i>et al.</i> , 2012)
6	Patrizia Falcone <i>et al.</i>	211 oocytes	2 hours	ICSI	71.1	2008	(Falcone <i>et al.</i> , 2008)

7	Li Xiao <i>et al.</i>	12,330 oocytes	3–4 hours	ICSI	85.37	2024	(Xiao <i>et al.</i> , 2024)
8	Yini Zhang <i>et al.</i>	57,632 oocytes	3–4 hours	ICSI	75.59	2020	(Zhang <i>et al.</i> , 2020)
9	Jingbo Chen <i>et al.</i>	246 oocytes	3–4 hours	ICSI	25.93	2023	(Chen <i>et al.</i> , 2023)
10	M. Carvalho <i>et al.</i>	506 oocytes	3–4 hours	ICSI	63.80 ± 23.00	2020	(Carvalho <i>et al.</i> , 2020)
11	Patrizia Falcone <i>et al.</i>	1228 oocytes	5 hours	ICSI	48.5	2008	(Falcone <i>et al.</i> , 2008)
12	Jason Yen-Ping Ho <i>et al.</i>	132 oocytes	4.5–5 hours	ICSI	50	2003	(Ho <i>et al.</i> , 2003)
13	M. Carvalho <i>et al.</i>	373 oocytes	4 hours	ICSI	67.00 ± 22.70	2020	(Carvalho <i>et al.</i> , 2020)

Results from studies differing in the timing of the extended denudation (2–5 hours) were aggregated in Table 2 to examine the influence of this on ICSI fertilization rates. It demonstrates an all-encompassing lack of consensus in the existing literature regarding an adequate or effective time frame for denudation. A range of fertilization rates in the period from 2 to 3 hours was reported, with an outlier low of 19.90% (Chen *et al.*, 2023) and a high of 71.10% (Falcone *et al.*, 2008). In the 3–4 hour follow-up interval, a similar trend was observed, demonstrating generally high success rates. For instance, success rates of 85.37% (Xiao *et al.*,

2024) and 75.59% (Zhang *et al.*, 2020) were reported, in contrast to the substantially lower rate of 25.93% reported by Chen *et al.* (2023). Denudation intervals approaching five hours do appear to decrease the actual fertilization rate—50.00% in Ho *et al.* (2003) and 48.50% in Falcone *et al.* (2008). This high variability strongly suggests that, despite denudation interval being a relevant issue, its impact could be more dependent on large heterogeneity in study design, patient population, or laboratory culture conditions, and requires more controlled studies to identify a consistent best extended denudation interval for the success of ICSI.

Table 3. Fertilization Rates According to Denudation Timing (5–8 hours)

Sr. No.	Author(s)	Sample Size	Denudation Time	Procedure	Fertilization Rate (%)	Year	References
1	Jason Yen-Ping Ho <i>et al.</i>	105 oocytes	>5.5 hours	ICSI	56.2	2003	(Ho <i>et al.</i> , 2003)
2	Patrizia Falcone <i>et al.</i>	173 oocytes	5 hours	ICSI	48.5	2008	(Falcone <i>et al.</i> , 2008)
3	Jingbo Chen <i>et al.</i>	246 oocytes	5–6 hours	ICSI	10.34	2023	(Chen <i>et al.</i> , 2023)
4	Patrizia Falcone <i>et al.</i>	223 oocytes	6 hours	ICSI	41.2	2008	(Falcone <i>et al.</i> , 2008)
5	Patrizia Falcone <i>et al.</i>	194 oocytes	7 hours	ICSI	34.7	2008	(Falcone <i>et al.</i> , 2008)
6	Patrizia Falcone <i>et al.</i>	212 oocytes	8 hours	ICSI	27.3	2008	(Falcone <i>et al.</i> , 2008)

The data from Table 3, on the impact of very late oocyte denudation timing (5–8 hours) on later ICSI fertilization

rates, shows a detrimental effect as time increases. In a different line of research, Ho *et al.* (2003) reported a rate of 56.20%

after 5.5 hours and beyond that was relatively stable; however, the majority of the evidence, in particular the extended series of findings from Falcone *et al.* (2008), is consistent. There is a clearly negative relationship between the length of time it takes an oocyte to denude and the success of fertilization. Specifically, Falcone's results show that fertilization rates actually decreased from 48.50% at five hours to 27.30% by eight hours, suggesting that retaining the oocyte longer with the associated cumulus cells upon retrieval substantially diminishes the ability of the oocyte to develop competently for successful fertilization by ICSI. Additionally, the extremely low rate of fertilization (10.34%) in Chen *et al.* (2023) in a group showing delays of five to six hours highlights the risk of failure that these prolonged slowdowns pose, reaffirming that denudation should occur within earlier time frames.

3.2. Overall Trends in Fertilization Rates

The data indicate different fertilization rates, and no one denudation time is ideal for the maximum fertilization rate. Studies published in the literature, such as Mizuno *et al.* (2018) and Xiao *et al.* (2024), reported high-yield fertilization rates (over 85%) for short- to middle-term denudation times (0 to 4 hours), whereas Chen *et al.* (2023) also found substantially lower fertilization rates over the same length of time. This inconsistency indicates that other determinants beyond denudation time, such as patient parameters, laboratory protocols, and oocyte quality, are crucial in fertilization success.

3.3. The Impact of Prolonged Denudation Time

The results indicate that prolonged periods of denudation have long-term adverse effects on fertilization rates. The reduced fertilization rate in some cases becomes obvious when the denudation phase exceeds 5 hours. A study by Falcone *et al.* (2008) showed that fertilization rates dropped from 48.5% at 5 hours to just 27.3% at 8 hours. This implies that although a brief pre-incubation time could aid oocyte maturation, an excessively prolonged wait may result in oocyte aging and negatively affect fertilization success.

3.4 Blastocyst Formation Rates

The present data show a major limitation due to the lack of data about blastocyst formation rates. The only two studies providing this critical metric were Düzgün *et al.* (2022) and Mizuno *et al.* (2018). Given the limited data from these studies, it is impossible to draw a definitive conclusion. This gap in research does not allow adequate evaluation of how denudation time affects embryo development, which is one of the most important factors in predicting pregnancy success.

4. Discussion

As illustrated in this literature review, selecting the desired denudation time duration for ART is a complicated process when determining the best duration for denudation in Assisted Reproductive Technologies (ART). The reviewed trials suggest that there can be no universal applicability of the denudation duration, with the duration being determined according to the particular technique used (ICSI), the exact timing of treatment, and other related aspects.

The studies reviewed provide a mixed picture of how the time at which denudation occurs impacts ART efficacy. Several studies, including those by Aletebi *et al.* (2011) and Mizuno *et al.* (2018), suggest that denudation occurring soon after retrieval may improve fertilization rates, recording an exceptionally high rate of 88.40% with immediate denudation. In contrast, the research conducted by Falcone *et al.* (2008) proposes that an enhanced clinical pregnancy rate is associated with a slightly extended pre-incubation period (5 hours), reflecting a hidden compromise between the instant fertilization effect and the long-term reproductive viability of the embryo. This observation supports the idea that a short interval of pre-incubation for the oocyte is essential for complete oocyte maturation to occur before ICSI without interruption.

Analysing the effect on blastocyst formation rates is critical and has not been extensively studied in the reviewed literature. There are indications from some reports, such as those by Mizuno *et al.* (2018) and Düzgün *et al.* (2022), which reported blastocyst rates; however, the available data are limited, making the conclusions less definitive. The study by Mizuno *et al.* indicated that the rate of high-quality blastocysts was lower with immediate denudation (52.0%) compared to a 2-hour incubation period (33.1%), although the sample sizes were small. This points to a significant deficiency in the existing literature and emphasizes the importance of conducting additional studies that monitor embryo development beyond the point of fertilization.

A central finding of this review was that prolonged denudation, or pre-incubation duration had a negative impact. Studies by Zhang *et al.* (2020) and Chen *et al.* (2023) found that the clinical pregnancy rate was lower when post-denudation and ICSI (DN-ICSI) waiting times exceeded 4 hours. Likewise, Falcone *et al.* (2008) noted a significant decrease in pregnancy rates when pre-incubation time exceeded 7 hours. This means that although some pre-incubation may be beneficial, extensive delay can degrade oocyte quality and embryo viability. Although the exact cause is not fully understood, it could be related either to oocyte aging or environmental stress.

Such conflicting results, similar to those reported by Naji *et al.* (2018), who observed no significant effect of denudation timing on outcomes, further complicate the development of a

universal protocol. Such variability might be due to differences in laboratory characteristics, patient populations, and study methodologies across studies. However, some studies focused on the duration from oocyte retrieval to denudation, whereas others focused only on the time between denudation and ICSI, or even between oocyte retrieval and ICSI. These different time metrics make direct comparison challenging.

In conclusion, although the data suggest a potential detrimental effect of excessively delayed denudation, the ideal time range remains elusive. The optimal denudation time appears to be a finely tuned trade-off between oocyte maturation and cellular degradation. More studies are needed to clarify these findings and to provide evidence-based best practice guidelines for embryologists worldwide.

5. Conclusion

The timing of denudation during ART procedures has a demonstrable impact not only on fertilization rates but may also affect subsequent embryo development and clinical outcomes. There is no consensus on a single “optimal” denudation time; different studies have made a wide range of recommendations.

Overall, current data suggest that while short or immediate denudation periods may contribute to high fertilization rates, a modest pre-incubation interval of two to four hours post-retrieval may affect overall success and clinical pregnancy rates significantly more. Moreover, the review provides strong evidence that a longer time interval between oocyte retrieval and ICSI, especially beyond four hours, may adversely affect clinical pregnancy outcomes.

Furthermore, this review identifies some important research gaps. Many empirical studies focus solely on fertilization rates, but very little data are available regarding the effect of denudation timing on blastocyst formation, embryo quality, or live birth rates. There is a need for future studies to integrate standardized protocols on the timing of denudation and to carefully follow all ART outcomes, from fertilization to live birth, in order to provide evidence for establishing clear, evidence-based guidelines for clinical practice. The relationship between denudation timing and long-term ART success may contribute significantly to improvements in patient care and treatment outcomes.

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Authorship Contribution

Ram Dayal designed the research topic and wrote the final version of the manuscript. Shalini assisted with the literature

search, reviewed the paper, and contributed to writing the manuscript. Both authors read and approved the final manuscript.

Ethical Approval

No ethical approvals were required, as this study is a review article based on previously published literature.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this review paper.

Declaration

The authors confirm that the manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. All authors have reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Data Availability Statement

No new data were generated or analyzed in this review article. All information discussed in this manuscript is based on previously published literature and cited references.

Figure Permission

No permission was required, as no copyrighted figures or images were used in this manuscript.

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