Effect of the time interval between oocyte retrieval and ICSI on embryo development and reproductive outcomes: a systematic review

Xue Wang, YaLing Xiao, ZhengYi Sun*, JingRan Zhen and Qi Yu

Abstract

Background: Intra-cytoplasmic sperm injection (ICSI) is used in assisted reproductive technology (ART) laboratories. However, there is no consensus regarding the precise time intervals within ICSI cycles (oocyte pick up (OPU), oocyte denudation (DN), and ICSI), and results are inconsistent and contradictory. Thus, we aim to evaluate whether there is a concordance regarding the time intervals used in different laboratories and a concrete time that gives better laboratory and reproductive results.

Methods: A systematic review of the literature until July 25, 2020, was performed with the keywords “Oocyte Denudation/Denudation/Oocyte,” “Intra-cytoplasmic Sperm Injection/ICSI,” “Oocyte/Oocyte maturation/ cumulus,” and “Cumulus removal/ removal.” Articles and abstracts in English and involving human subjects referring to the effects of oocyte DN time on embryo development and clinical outcomes were included.

Results: Of the 294 evaluated articles, 24 (including 20 full articles and 4 abstracts) were included in this review. Eighteen studies analysed the effect of OPU-DN time on embryo development and clinical outcomes. Most of these studies concluded that OPU-DN time did not influence ICSI outcomes, but some suggested that oocytes should be incubated for a short time before DN to improve oocyte maturity and enhance ICSI outcomes. In addition to reports on positive or negligible effects, adverse effects were reported in 12 studies on DN-ICSI timing. Neither OPU-DN nor DN-ICSI time could improve live birth rate.

Conclusions: Oocytes should be pre-incubated for a short duration (preferably < 4 h) before DN according to the ART laboratory schedule. More randomised controlled trials are warranted to clarify the effect of DN-ICSI timing on ICSI outcomes.

Keywords: Oocyte denudation, ICSI, Fertilisation rate, Embryo development, Clinical pregnancy rate

* Correspondence: sunzhengyi@263.net
Department of Gynecology Endocrine and Reproductive Center, Peking Union Medical College Hospital, Peking Union Medical College/Chinese Academy of Medical Sciences, Beijing 100730, China

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Background

Intra-cytoplasmic sperm injection (ICSI) is a technique to achieve fertilisation for couples with severe male infertility, in which the sperm is inserted into the cytoplasm of the oocyte [1]. After oocyte collection, the cumulus cells need to be denudated before ICSI, a process called oocyte denudation (DN). However, different assisted reproductive technology (ART) laboratories implement different protocols depending on their daily workload and different time intervals, including the time of oocyte pick up (OPU), DN, and ICSI [2–5].

During follicular development, cumulus cells that surround the oocytes can promote maturation of cytoplasm and nucleus of the oocytes through an autocrine/paracrine mechanism and communicate through gap junctions [6, 7]. Before ovulation, cumulus cells proliferate widely and provide basic nutrition for oocytes to support their development. Additionally, cumulus cells also participate in meiotic arrest, transcription regulation, and induction of cytoplasmic maturation [8, 9]. After ovulation, oocytes resume meiotic separation under Luteinizing Hormone (LH) stimulation; they complete the first meiosis, stop at the second meiosis metaphase (MII), and reach meiotic maturation [10]. Simultaneously, cumulus cells produce hyaluronic acid and expand in vitro, and further induce oocyte cytoplasmic maturation by stimulating gene expression and reducing oxidative stress [11, 12]. Research shows that after ovulation induction, gap junctions between cumulus cells around oocytes continue to exist, whereas distant junctions disappear. Using this strategy, intact cumulus cells can use gap junctions between oocytes to promote oocyte maturation [13]. However, DN must be performed prior to ICSI for two reasons: a) the cumulus cells affect the entrance of the injection needle, and that a cumulus-oocyte-complex (COC) cannot be held properly by the holding pipette; b) oocyte maturity must be assessed since only mature oocytes that have reached the MII stage should be injected. Moreover, the polar body has to be placed at 12 or 6 o’clock positions to avoid the oocyte spindle from being damaged [14].

Therefore, an important question arises: When should the cumulus cells around the oocytes be removed to obtain the optimal ICSI outcome? Though many researchers have tried to confirm the optimal timing intervals in ICSI procedures, these time intervals and their impact on the outcomes of ICSI cycles remain controversial. Some studies have found that prolonging the incubation periods between oocyte pickup (OPU) and DN can improve the rates of fertilisation and blastocyst formation, which is conducive to embryo development. It is suggested that oocytes should be incubated for a few hours before DN [15–17]. However, recent studies in mice have shown that prolonged incubation time before DN can induce oocyte apoptosis. In contrast, some studies suggest DN immediately after oocyte retrieval [18]. Other studies found that prolonging the time of OPU-DN could not improve the rates of fertilisation or clinical pregnancy [5, 19]. A negative correlation between the time of DN and ICSI has also been reported, i.e., longer DN-ICSI time lowers the fertilisation rate, and may lead to adverse pregnancy outcomes [17, 20]. Therefore, the aim of this study was to systematically analyse and review the published literature on the impact of time intervals on embryo development and clinical outcomes and determine the optimal time intervals during ICSI cycles.

Materials and methods

PubMed and the Embase databases were searched for relevant studies and reviews using the following keyword combinations: 1) ‘Oocyte Denudation/Denudation/Oocyte’ and ‘Intra-cytoplasmic Sperm Injection/ICSI’ and 2) ‘Oocyte/Oocyte maturation/ cumulus’ and ‘Cumulus removal/ removal.’ The search strategy was limited to articles published in English involving human subjects, with the last search performed on 25 July 2020. Articles that described the effect of DN time on embryo development and clinical outcomes were included in this study, with the additional requirement that they should include at least one of the following indicators: mature egg rate, fertilisation rate, high-quality embryo rate, cleavage rate, blastocyst formation rate, implantation rate, clinical pregnancy rate, and live birth rate. The specific selection process is shown in Fig. 1.

Results

Design and methodology of the studies

A total of 294 articles were identified in our initial search, of which 24 (four abstracts and twenty articles) were selected for this review. Among the twenty-four studies, sixteen were retrospective, and only eight were prospective. The characteristics of the studies that we included in the relationship between oocyte DN time and ICSI outcomes are presented in Table 1. Two of these studies utilised donor oocytes [19, 28], and the study of Bárcena et al. [19] included both fresh and freeze-thawed oocytes. Therefore, to avoid the impact of freezing on oocytes, only fresh oocyte data from their study were included. The other 22 studies used autologous oocytes from patients themselves. Three abstracts did not mention the ovarian stimulation protocol [27, 28, 30]. Of the other 21 studies, those prior to 2011 used GnRH agonist protocol (GnRHa). Later studies used GnRHa and GnRH antagonists (GnRH ant) except for one that only used GnRH ant. Additionally, there has been one study on natural cycles and micro-stimulation protocols [29]. Five studies did not mention the time
from human chorionic gonadotropin (HCG) administration to oocyte collection. Most of the other studies used approximately 36 h, with the shortest time being 34 h [5, 26, 35], and the longest time being more than 39 h [34]. Four studies recorded the time with an automatic radiofrequency-based system (RI-Witness, Research Instruments, UK) [19, 20, 32, 35], and the others recorded the time manually. Of the 24 studies, 14 did not mention the environment in which oocytes were incubated, while 7 mentioned 20% O₂, and only 3 used 5% O₂ [15, 32, 35]. Eighteen studies analysed the effect of OPU-DN time on embryo development or pregnancy outcomes. Patrat et al. [17] analysed an interval of 0.5 h, whereas Pujol [20] and Bárcena et al. [19] were grouped according to a decile. Six of the studies conducted ICSI immediately after DN. One study was grouped according to HCG-DN [35]. Twelve studies analysed the influence of DN-ICSI time on the outcomes; however, in one study that used a fixed DN time, objects were grouped by OPU-ICSI time [24].

**Impact of OPU-DN on oocyte maturation rate**
The oocyte maturation rate was analysed in 16 studies (Table 2), and 11 studies suggested that prolonging OPU-DN time did not increase the oocyte maturation rate. The other five studies concluded the opposite trend, and one of them found that oocyte maturation was remarkably lower with immediate degranulation than with 4-h incubation with intact cumulus 80.5% vs. 91.9%) [2]. A considerable difference in the oocyte maturation rate was also found between the 2-h and 4-h incubation periods (78.40% vs. 84.81%) [28].

**Impact of OPU-DN on fertilisation rate and embryo development**
Seventeen studies reported data on the effect of OPU-DN on fertilisation rate. Among them, 11 reported no significant effect of OPU-DN time on fertilisation rate, which was similar to the conclusion of a recent study, suggesting no correlation between OPU-DN time and fertilisation rate [33]. However, six studies considered that OPU-DN time did affect fertilisation rate, and five of these believed that the fertilisation rate would increase with the extension of OPU-DN time [2, 4, 15, 17, 23]. Another study found that the fertilisation rate was the highest when oocytes were degranulated within 2 h, compared with that at 2–3 h or longer duration (91.2% vs. 87.3% vs 82.0%, respectively) [29].
The impact of OPU-DN time on embryo development and subsequent embryo quality was evaluated by 11 studies. Among them, six reported no effect on embryo quality. One study was divided into 1–3 h, 3–5 h, 5–7 h, 7–9 h, and 9–11 h groups according to OPU-DN time, and showed that embryo quality that was similar in the first four groups but remarkably higher than that in the fifth group [3]. The remaining study showed a significantly increasing rate of good-quality embryos with extended OPU-DN. Regarding blastocyst formation, two
studies found a trend for this to be more frequent for the 2-h or 4-h incubation periods with intact cumulus cells than with immediate degranulation, but the differences were not significant [2, 16].

Impact of OPU-DN on implantation rate and clinical outcome
A total of seven studies analysed the effect of OPU-DN time on implantation rate. Among them, six concluded that implantation rate was not affected by OPU-DN time, while the remaining one found that a group subjected to 1.5–2 h oocyte incubation showed a strikingly higher implantation rate than the other groups [17]. Among the 16 studies on the effect of OPU-DN on pregnancy rate, 15 studies suggested that prolonging OPU-DN time did not lead to any improvement. However, one study found that, compared with the immediate DN group, the clinical pregnancy rate was significantly increased with 1 h or 2 h oocyte incubation prior to DN [2, 4]. The data of the five studies on the effect of OPU-DN time on the living birth rate revealed that there was no significance among groups [16, 17, 19, 20, 32].

Impact of DN-ICSI on oocyte maturation
Four studies mentioned the effect of DN-ICSI time on the rate of mature oocyte production, three of which found that prolonging DN-ICSI did not improve oocyte maturation [2, 17, 21] (Table 3). One study suggested that prolonging the incubation time before ICSI could promote oocyte maturation, but it was unclear whether the effect originated from the incubation before or after degranulation [4].

Impact of DN-ICSI on fertilisation rate and embryo development
In terms of fertilisation rate, seven studies considered that DN-ICSI time had no effect on the fertilisation rate, whereas the other five had the opposite conclusions. One study found that the fertilisation rate was negatively correlated with DN-ICSI time (0–3 h) [17]. Another study showed that the fertilisation rate was stable within 6 h after degranulation, and the stability decreased remarkably after 6 h [3], while two others suggested that prolonging the time of DN-ICSI could increase the fertilisation rate [4, 20]. However, one recent study that grouped subjects according to DN-ICSI time found that, although the fertilisation rate gradually increased with incubation times up to 5 h, it decreased considerably after 5 h [33].

Four of the five studies revealed that there was no effect of DN-ICSI time on embryo development. One study showed a non-significant decreasing trend in the frequency of good-quality embryos when the DN-ICSI was more than 5 h [33]. However, another study found more good-quality embryos in a group incubated for 3 h after DN compared to a group with immediate ICSI.

### Table 2 Effect of OPU-DN time on oocyte maturation and ICSI outcome

| Author               | Compared times(h) | MOR (%) | FR (%) | GER (%) | BFR (%) | IR (%) | PR (%) | LBR (%) |
|----------------------|-------------------|---------|--------|---------|---------|--------|--------|---------|
| Velde et al. (1998) [21] | 1–2 vs 5–6        | N       | N      | N       | ND      | ND     | ND     | ND      |
| Yanagida et al. (1998) [22] | 1–3 vs 3–5 vs 5–7 vs 7–9 vs 9–11 | N       | N      | Y       | ND      | ND     | N      | ND      |
| Rienzi et al. (1998) [23]  | ≤3 h vs 3–6 vs 6–9 vs 9–12 | N       | Y      | Y       | ND      | N      | N      | N       |
| Hassan et al. (2001) [2]   | 0 vs 4            | Y       | Y      | ND      | N       | ND     | N      | ND      |
| Jacobs et al. (2001) [25]  | 0–2 vs 2–3 vs 3–4 vs > 4 | N       | N      | N       | ND      | N      | N      | ND      |
| Ho et al. (2003) [26]      | < 2.5 vs 2.5–3.5 vs 3.5–4.5 vs 4.5–5.5 vs ≥5.5 | Y       | N      | N       | ND      | ND     | N      | ND      |
| Isiklar et al. (2004) [15] | 0 vs 2–4          | Y       | Y      | N       | ND      | N      | N      | ND      |
| Aletebi (2011) [4]         | 0 vs 1 vs 2       | Y       | Y      | ND      | ND     | N      | N      | ND      |
| Patrat et al. (2012) [17]  | 0 vs 0.5 vs 1 vs 1.5 vs 2 vs 2.5 vs 3 | N       | Y      | N       | ND     | Y      | N      | N       |
| Eibert et al. (2013) [28]  | 2 vs 4            | Y       | N      | N       | ND      | N      | N      | ND      |
| Garor et al. (2015) [5]    | < 2 vs > 2        | ND      | N      | ND      | ND     | N      | N      | ND      |
| Terasawa et al. (2016) [29] | < 2 vs 2–3 vs > 3 | ND      | Y      | ND      | ND     | ND     | N      | ND      |
| Ishikawa et al. (2016) [30] | 0 vs 2           | N       | N      | Y       | N       | ND     | ND     | ND      |
| Bárcena et al. (2016) [19] | 3 h by deciles   | ND      | N      | N       | ND      | ND     | N      | N       |
| Pujol et al. (2018) [20]   | 2.12 h by deciles | ND      | ND     | ND      | ND     | ND     | N      | N       |
| Mizuno et al. (2018) [16]  | 0 vs 2            | N       | N      | N       | Y       | N      | ND     | N       |
| Naij et al. (2018) [32]    | 0 vs 0–2 vs 2–5   | N       | N      | ND      | ND     | N      | N      | N       |
| Azizi et al. (2020) [34]   | < 2 vs > 2        | N       | N      | ND      | ND     | N      | N      | ND      |

Y Yes, N No, ND Not Done, MOR Mature Oocyte Rate, FR Fertilisation Rate, GER Good Embryo Rate, BFR Blastocyst Formation Rate, IR Implantation Rate, PR Pregnancy Rate, LBR Live Birth Rate
(37.3% vs. 27.9%; p < 0.05) [27]. Only one study on the blastocyst formation rate found that this did not differ significantly between a group incubated for 4 h after DN and a group without incubation after DN (16.7% vs. 18.8%) [2].

**Impact of DN-ICSI on implantation rate and clinical outcome**

Three studies analysed the effect of DN-ICSI time on implantation rate. One study found that DN-ICSI time (within 3 h) did not affect implantation rate [17]. One study reported that the implantation rate was 24.6% in the immediate ICSI group, which was considerably higher than that in the 1–3-h incubation group (15.5%) [27]. Another study found that the results varied with different cut-off values of DN-ICSI time. When the cut-off value was 2 h, the implantation rate was strikingly higher in the < 2-h group than in the > 2-h group, but there was no significant difference between the two groups when the cut-off value was 3 or 4 h [33].

A total of ten studies analysed the effect of DN-ICSI time on pregnancy outcomes, half of which indicated that prolonging DN-ICSI time did not improve the clinical pregnancy rate, whereas the other five suggested that DN-ICSI time had an effect. One study found that prolonging DN-ICSI interval duration could improve the clinical pregnancy rate [4], whereas another found that the pregnancy rate increased gradually for the first 6 h, but decreased remarkably afterward [3]. In contrast, one study suggested that longer DN-ICSI duration lowered the clinical pregnancy rate, which found that each 1-h increase in DN-ICSI time reduced the likelihood of clinical pregnancy by 7.9% [14]. Recently, Zhang et al. [33] reported that the clinical pregnancy rate did not vary with DN-ICSI times less than 4 h, but did decrease notably over 4 h. There was no statistical difference in the four studies that analysed the effect of DN-ICSI time on live birth rate [17, 19, 20, 24]. The results and conclusions are listed in Table 4.

**Discussion**

For ART to achieve a positive outcome, it is important to obtain high-quality mature oocytes. Oocytes maturation involves both nuclear and cytoplasmic maturation [36]. Nuclear maturation involves recovery from the first meiosis, germinal vesicle breakdown, and the first polar body formation. Cytoplasmic maturation can help prepare the oocyte for fertilisation and subsequent embryonic development, and it can provide enough energy, enzymes, and protein synthesis reserve to meet the needs of new functional protein synthesis during embryonic development [8, 9]. Unlike the nucleus, there is no clear standard for defining and detecting cytoplasmic maturation, which is a highly complex process. In the natural process, the cytoplasm and nucleus may mature synchronously in some way, but they may not be completely synchronised in the ovulation induction cycle [21]. If the cytoplasm of oocytes is not mature during ICSI, this may directly affect fertilisation and embryo development. It may also impair the supply of material to the embryo, resulting in early embryo death and pregnancy failure. Whether intact or incomplete cumulus cells should be incubated before ICSI remains controversial. Therefore, this issue is discussed in the following sections.

After the introduction of ICSI, based on experience with in vitro fertilisation, some researchers hypothesised that incubation of the obtained oocytes before ICSI for a certain duration before DN and/or ICSI might help to achieve a better outcome. However, to date, no
| Study                     | Items            | Results                                                                                           | Conclusions                                                                                           |
|--------------------------|------------------|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| Velde et al. (1998) [21] | OPU-DN           | No difference in MOR, FR, and GER between groups                                                   | ICSI should be delayed until the noon hour to observe fertilization in the following morning          |
|                          | DN-ICSI          | No difference in MOR, FR, and GER between groups                                                   |                                                                                                       |
| Yanagida et al. (1998)  | OPU-DN           | The MOR, FR, CR, and PR were similar between groups. When incubated for > 9 h, the MOR was significantly decreased, while the PR showed a downward trend | ICSI is performed at any time within 1–9 h following oocyte retrieval                                   |
| Rienzi et al. (1998)    | OPU-DN           | No differences in MOR, CR, and PR between groups. The FR and GER of the group with incubation time of < 3 h were significantly lower than those with incubation time of > 3 h | The optimum time range between 3 and 12 h following oocyte retrieval can improve the fertilization rate and embryo quality |
| Andrews et al. (2001)   | OPU-ICSI         | No differences in FR and LBR. The CR of the group incubated for < 3 h was superior to that incubated for > 5 h | The shorter incubation durations (< 3 h) generally seemed to produce better results than longer ones (> 5 h) |
| Hassan et al. (2001)    | OPU-DN           | The MOR, FR, BFR, and PR were significantly higher in the 4-h incubation group than those in the immediate DN group | Aspects of nuclear, cytoplasmic maturation, and oolemma properties were improved when oocytes were preincubated with intact cumulus before DN rather than after DN |
| Jacobs et al. (2001)    | OPU-DN           | No significant differences in FR, GER, IR, and PR among all groups                                  | Incubation durations of 30 min to 6 h prior to ICSI did not improve the ICSI results. Should ICSI are needed in advance, no incubation is needed. |
| Ho et al. (2003)        | OPU-DN           | The MOR in the group with an incubation time of < 2.5 h was significantly lower than that of > 2.5 h. No differences in FR, GER, and PR among all groups. | Nuclear maturity of the oocytes with hCG administration 34 h earlier could be increased by incubation for 2.5 h before DN |
| Isiklar et al. (2004)   | OPU-DN           | The MOR, FR, and GER of 2–4 h incubation group before DN were significantly higher than those of no incubation group. However, no differences in IR and PR among the two groups | Pre-incubation of oocytes prior to ICSI is associated with improved maturation of oocytes, fertilization, and embryo quality |
| Falcone et al. (2008)   | DN-ICSI          | With the extension of OPU-ICSI, the FR and CR were gradually increased, peaking at 5–6 h (3–4 h for DN-ICSI), and then gradually decreased with the extension of OPU-ICSI | The most appropriate incubation time for mature oocytes before ICSI is 5–6 h |
| Boldi et al. (2010)     | DN-ICSI          | No significant difference in FR between the two groups, however the GER, IR, and PR in the group within 1 h of DN-ICSI were significantly higher than those with 1–3 h of DN-ICSI | Oocytes should be injected as soon as possible following cumulus removal to improve the ICSI outcome |
| Aletebi (2011)          | OPU-DN           | The MOR and FR of the 2-h group were significantly higher than those of the 1-h group; no incubation group before and after DN. The PR of the 2-h group and 1-h group was similar and significantly higher than that of the no incubation group | It is preferable to allow an interval between oocyte retrieval and sperm injection                      |
| Patrat et al. (2012)    | OPU-DN           | The total time of OPU-DN within 3 h had no effect on the MOR and GER. The FR gradually increased, the IR peaked at 1.5–2 h, and the PR reached a high value at approximately 2 h | Incubation of oocytes approximately 2 h before DN may not increase MOR; however may lead to the optimal combination of FR and IR. Meanwhile, the sperm injection should be achieved without any delay following oocyte denudation to maintain good fertilization results |
|                         | DN-ICSI          | DN-ICSI had no effect on MOR, GER, IR, and PR; however, FR gradually decreased within 3 h.        |                                                                                                       |
Studies have confirmed that appropriate prolongation of founding factors, one of which was the HCG-OPU time. Among these five studies, two suggested that prolonging the HCG-OPU and OPU-DN time was beneficial to oocyte cytoplasmic maturation \([26, 31]\). Ho et al. \([26]\) described that the HCG-OPU and OPU-DN time was approximately 36 h, with a better homogeneity. In this review, we found in vivo maturation time, i.e. HCG-OPU > 36 h, can improve oocyte maturation, thereby improving embryo and pregnancy outcomes \([37, 38]\). In this review, we found that the HCG-OPU time in five studies was less than 36 h \([5, 26, 31, 34, 35]\). However, in other studies, HCG-OPU was approximately 36 h, with a better homogeneity. Among these five studies, two suggested that prolonging the HCG-OPU and OPU-DN time was beneficial to oocyte cytoplasmic maturation \([26, 31]\).

### Table 4 The results and conclusions of the 24 studies included in the review (Continued)

| Study | Items | Results | Conclusions |
|-------|-------|---------|-------------|
| Eibert et al. (2013) \([28]\) | OPU-DN | Compared with the 4-h incubation group before DN, the MOR in the 2-h incubation group was significantly lower, while the FR, GER, IR, and PR remained unchanged. | Delaying denudation procedure resulted in a higher MOR, although did not influence clinical outcomes |
| Garor et al. (2015) \([5]\) | OPU-DN | No impact on the FR and PR regardless of the HCG–OPU interval. | Delaying oocyte denudation or sperm injection did not compensate for insufficient exposure to the follicular environment after hCG triggers before ovulatory oocyte maturation. |
| | DN-ICSI | No impact on the FR and PR regardless of the HCG–OPU interval. | |
| Terasawa et al. (2016) \([29]\) | OPU-DN | The FR in the DN group within 2 h of OPU was significantly higher than that in DN group of > 2 h | Cumulus-oocyte complexes are recommended to be denuded soon after OPU in the case of ICSI |
| Ishikawa et al. (2016) \([30]\) | OPU-DN | No significant differences in the MOR, FR, and BFR between the two groups, however, the rate of high-quality blastocysts was significantly increased after 2 h of incubation | Oocyte culturing with cumulus cells for 2 h or longer improved the resulting blastocyst quality |
| Pereira et al. (2016) \([31]\) | OPU-DN | The time of OPU-DN in the fertilization group was significantly longer than that in the non-fertilization group | Modulating time intervals between OPU, DN, and ICSI to grant fertilization seems feasible |
| Bárcena et al. (2016) \([19]\) | OPU-DN | No differences in FR, GER, PR, and LBR among all groups | High-quality oocytes may withstand ageing in vitro to a certain extent, allowing for easier planning of laboratory workflow without a detrimental effect on the outcomes |
| | DN-ICSI | No differences in FR, GER, PR, and LBR among all groups | |
| Pujol et al. (2018) \([20]\) | OPU-DN | No effect on PR, continued pregnancy rate and LBR | The PR diminishing was progressive as time OPU-ICSI increases. ICSI should not be delayed whenever possible. |
| | DN-ICSI | The PR decreased by 7.9%, when the DN-ICSI time was increased by 1 h, with no effect on LBR. | |
| Mizuno et al. (2018) \([16]\) | OPU-DN | No significant differences in MOR, FR, BFR, PR, and LBR between the two groups, but the high-quality blastocyst rate was significantly increased | Intact cumulus cells should be maintained during the preincubation period, since they are essential to embryonic development post fertilization |
| Naji et al. (2018) \([32]\) | OPU-DN | No significant difference, in MOR, FR, BFR, IR, PR, and LBR between different groups | Oocyte denudation within 2 h or 2–5 h results in a comparable outcome, permitting more efficiency and flexibility in scheduling laboratory workload |
| Zhang et al. (2020) \([33]\) | DN-ICSI | The FR increased with longer DN-ICSI interval within 5 h and declined with DN-ICSI interval > 5 h. The PR was significantly higher in DN-ICSI interval of < 4 h compared to that of > 4 h | The optimal time for ICSI is within 4 h following oocyte denudation for excellent outcomes in ICSI cycles |
| Azizi et al. (2020) \([34]\) | OPU-DN | Be was associated with cytoplasmic granulation and extended PVS of oocytes | The time intervals in the ICSI cycle alters oocyte quality, with no significant impact on the reproductive outcomes |
| | DN-ICSI | The DN-ICSI was associated with oocytes cytoplasmic granulation. The FR, CR, and PR were not associated with the time intervals in ICSI cycles. | |
| Maggiulli et al. (2020) \([35]\) | IO-DN | No effect on MOR, however, the BFR was decreased while IO-DN increased. | IO-DN did not affect the cumulative live birth rate, but affected the BFR. |

**IO** Induction of ovulation, **MOR** Mature Oocyte Rate, **FR** Fertilisation Rate, **CR** Cleavage Rate, **GER** Good Embryo Rate, **BFR** Blastocyst Formation Rate, **IR** Implantation Rate, **PR** Pregnancy Rate, **LBR** Live Birth Rate
found that when HCG-OPU was 34 h, the oocyte maturity in the group that were degranulated within 2.5 h was significantly lower than that of other groups, with no differences in fertilization rate and pregnancy outcome. And they suggested that prolonging HCG-OPU time could shorten in vitro incubation [26]. Maggiulli et al. [35] found no correlation between HCG-OPU and mature egg rate and embryo development. Two studies had divided groups into either HCG-OPU < 36 h or > 36 h and found the time of OPU-DN had no effect on fertilization and clinical pregnancy rates regardless of whether HCG-OPU was more or less than 36 h [5, 34]. Therefore, for good homogeneity, we only collected data in groups with HCG-OPU > 36 h in the two studies.

A study has found evidence that meiosis of oocytes might be blocked by the corona-cumulus-complex, suggesting that oocytes could complete meiosis after the removal of cumulus cells [23]. However, almost all the included studies have concluded that DN-ICSI time does not affect oocyte maturation. Hassan [2] compared the effects of OPU-DN and DN-ICSI on oocyte maturation rate, and found that incubation with intact cumulus cells before DN could improve the maturation of oocytes, whereas incubation after DN could not improve the maturation of oocytes. Some studies have suggested that oocyte regulation and the consequent gene expression of cumulus cells and bidirectional control require expression gap junction-associated genes and structural integrity [39]. The expression of connexin and gap junctions was related to oocyte maturation after a short time but decreased with oocyte maturation [40]. Therefore, the role of cumulus cells in controlling oocyte maturation is significant before germinal vesicle breakdown. After ova-

lination induction, gap junctions between cumulus cells around oocytes continue to exist, whereas distant junctions disappear. Using this strategy, intact cumulus cells can use gap junctions between oocytes to promote oocyte maturation [13]. The specific mechanism underlying this process needs further research.

There is no consensus on the effect of OPU-DN on fertilisation rate; most studies have concluded that there is no significant effect. It has been suggested that this may be due to the synchronous development of the nucleus and cytoplasmic maturation of oocytes during ovulation induction rather than the beneficial effect of peripheral cumulus cells, or that the ICSI may have avoided some mechanisms associated with cytoplasmic maturation [21, 35]. Bárzana et al. [19] have suggested that oocytes from young and fertile people are more tolerant to long-term incubation in vitro without affecting their subsequent development. Patrat et al. [17] suggested that even if the number of mature oocytes could not be increased, incubation would further promote the cytoplasmic maturation of oocytes, improving their fertilisation potential. Peripheral cumulus cells may secrete some paracrine substances and growth factors or express some adhesion molecules to promote the nuclear or cytoplasmic maturation of oocytes [7, 39]. For example, brain-derived neurotrophic factor secreted by cumulus cells is important for the development of the nucleus and cytoplasm of oocytes [41].

Conflicting results concerning the effect of DN-ICSI time on fertilisation were found in the literature. Most authors have concluded that there is no effect, but Patrat et al. [17] have found that DN-ICSI time is negatively related to fertilisation rate. Another study has suggested that a longer incubation time results in spindle instability and chromosomal material loss in oocytes [42]. Therefore, the authors suggested that ICSI should be carried out immediately after degranulation. Some studies found that it was positively correlated with fertilisation rate but significantly decreased from 5 h after degranulation [33]. Some studies suggest that the reason for the increase in fertilisation rate is not only related to the further maturation of cytoplasm, but is also related to oocyte ageing [20, 33]. Oocyte ageing is related to the activities of the M-phase promoter (MPF) and mitogen-activated protein kinase (MAPK), which are important regulators of the second meiosis [43]. With the ageing of oocytes, the activities of MPF and MAPK decrease, which leads to the spontaneous activation of oocytes [44]. Therefore, ageing oocytes with less MPF levels are more likely to be activated by ICSI to form the pronucleus, thus explaining the increase in the fertilisation rate with increased OPU-ICSI time [20].

There is a special situation that cannot be ignored, oocyte spontaneous activation, which is a rapid and uncontrollable process. Normally, oocytes are obtained naturally or COH remains arrested at MII until fertilization, at which the oocyte resumes meiosis [45, 46]. Meiotic arrest is achieved through a series of cyto-

static factor activities, such as c-Mos/ MAPK and EMI2 [46–48]. However, in certain cases, advanced maternal age, high FSH exposure for a long time in COH, or high vacuum pressure during oocyte retrieval, may induce oocyte spontaneous activation [49–51]. Oocyte spontaneous activation can lead to premature separation of sister chromatids which are then scattered in the cytoplasm. Once re-activated by a sperm, these separated or scattered chromatids will form 3PN or multiple pronuclei (MPN) [45, 52]. Repeated abnormal fertilization has been previously reported, which may be caused by genetic defect resulting in spontaneous activation [45, 50, 51, 53, 54]. Additionally, the unexplained infertility or repeated pregnancy loss may also be associated with the parthenogenesis caused by spontaneous activation of oocytes prior to ovulation [45, 54]. Therefore, for those who failed to conceive after multiple cycles, especially
ably higher than that in the former three groups [3]. The were incubated for more than 5 h [27]. If the oocytes
were performing ICSI immediately after DN was higher than
found that the rate of high-quality embryos in the group
incubated for 3 h [32, 35], while
performing ICSI immediately after DN was higher than,
that in the immediate degranulation group with no incubation, significant differences were
not found [2, 16]. One recent study reported the effect of ICSI immediately after degranulation and after four
HCG-DN periods, namely < 37 h, 37–37.5 h, 37.5–38 h,
and > 38 h, and found that the blastocyst formation rate
decreased with time (44.6% ± 27.5, 39.8% ± 27.2, 36.9% ±
28.4, and 33.0% ± 27.8%, respectively) [35]. They sug-
gested that this was unrelated to the in vitro maturation
of immature oocytes, and that the specific mechanism
should be studied further [35].

Most of the studies examined for this review have con-
cluded that OPU-DN does not affect the implantation
and pregnancy rates. Some found that the highest
implantation rate was achieved with 1.5–2 h incubation be-
fore DN, a duration that also optimised the pregnancy
and live birth rates [17]. The clinical pregnancy rate fol-
lowing incubation for 1, 2, or 4 h before DN was higher
than that in the immediate degranulation group [2, 4]. It
was suggested that the reason for this could be the ap-
pearance of spindles, which resulted from further matur-
ation of the oocyte cytoplasm through the incubation of
cumulus cells [4]. It has been confirmed that if the spin-
dles in oocytes are visible upon observation, most of
which appears 39–40.5 h after hCG administration, the
fertilisation and implantation rates are higher, but after
this duration, the oocyte begins to deteriorate. There-
fore, it is recommended that ICSI should be performed
39–40.5 h after hCG administration [63, 64]. Consider-
ing the effects of the time of DN-ICSI on the pregnancy
outcome, Pujol et al. [20] found that, for each 1 h in-
crease in DN-ICSI time, the biochemical and clinical
pregnancy rates decreased by 7.5 and 7.9%, respectively,
but the continuous pregnancy and live birth rates were
unaffected. Moreover, they suggested in vitro ageing of
human oocytes significantly affected the chance of be-
coming pregnant, and ICSI should not be delayed when-
ever possible [20]. Two studies found that oocytes
incubated for 4 h or 6 h resulted in a similar or increased
pregnancy rate, which then decreased remarkably [3, 33].
This may be due to the oocytes reaching their best
states for fertilisation after incubation within a certain
duration before DN. Over-incubation of the oocyte can
lead to changes in ultrastructure and gene expression,
and increase the incidence of spindle abnormalities,
which will affect subsequent embryonic development
and reduce the clinical pregnancy rate [63, 65]. Cur-
rently, there is no indication that DN time affects the
live birth rate.

those who repeat 3PN or MPN, the intervals in ICSI
should be avoided since oocyte spontaneous activation
could be a contributing factor. To minimize the effect,
oocytes should be denuded immediately after collection,
followed by a careful and rapid ICSI to mitigate oocyte
spontaneous activation-induced abnormal fertilization
and possible aneuploidy.

More than half of the studies examined concluded that
prolonging OPU-DN time could not improve the rate of
good-quality embryos. Some studies found that incuba-
tion for 2–4 h before DN could increase the rate of
high-quality embryos [15, 16], but that longer times led
to a decrease in the rate of high-quality embryos [22].
Underlying reasons include the possibility that cumulus
cells may promote the further maturation of oocyte
cyttoplasm, conducive to subsequent embryonic develop-
ment. Conversely, COC is sensitive to oxidative stress
in vitro, and reactive oxygen species (ROS) can acceler-
ate oocyte ageing, which decreases MAPK activity,
meiosis acceleration, the non-separation of sister chrom-
atics, and the increase of aneuploid chromosome number in oocytes [55, 56]. Studies have shown that cu-
mulus cells can secrete glutathione, which may delay the
oocyte ageing process and improve embryo quality in a
short-term incubation [57, 58]. However, if incubation is
prolonged, the antioxidants produced by cumulus cells
are not enough to counteract ROS. Therefore, long-term
accumulated oxidative stress damages mitochondrial
DNA and reduces the ratio of ATP and glutathione/glutathione disulfide in cells, resulting in abnormal cyto-
skeleton fibres and calcium signalling in the endoplasmic
reticulum. It can also induce abnormal calcium oscilla-
tion after fertilisation, which manifests as abnormal fer-
tilisation and embryo development [59–61]. One study
found that the rate of high-quality embryos in the group
performing ICSI immediately after DN was higher than
that in the group incubated for 3 h [27]. If the oocytes
were incubated for more than 5–6 h, the frequency of
high-quality embryos decreased, but the frequency of in-
ferior embryos in the latter three groups was consider-
ably higher than that in the former three groups [3]. The
authors suggested that the results indicated that the
quality of embryo segmentation depended on the period
of oocyte preincubation before injection. In this review,
10 articles had mentioned culture environment, of which
only 3 had oocyte incubation at 5% O2 [15, 32, 35], while
most incubated at 20% O2. It has been confirmed that
20% O2 can accelerate the formation of ROS in culture
medium, induce histone modification, inactivate en-
zymes, and cause membrane lipid peroxidation, thereby
damaging the surface of embryo membrane and affecting
its development [56, 62]. Therefore, the obtained oocytes
should be incubated in hypoxia environment, which can
effectively reduce the production of exogenous ROS and
improve the embryonic development potential and out-
come of assisted reproduction.

There are a few studies on blastocyst formation rates.
Although two studies found that the blastocyst forma-
tion rate in the group incubated for 2 or 4 h after DN
was higher than that in the immediate degranulation
group with no incubation, significant differences were
not found [2, 16]. One recent study reported the effect
of ICSI immediately after degranulation and after four
HCG-DN periods, namely < 37 h, 37–37.5 h, 37.5–38 h,
and > 38 h, and found that the blastocyst formation rate
decreased with time (44.6% ± 27.5, 39.8% ± 27.2, 36.9% ±
28.4, and 33.0% ± 27.8%, respectively) [35]. They sug-
gested that this was unrelated to the in vitro maturation
of immature oocytes, and that the specific mechanism
should be studied further [35].
Conclusions
According to our literature review on the effects of the time intervals on embryo development and pregnancy outcomes, the results showed that the incubation time before degranulation usually had a small positive effect on ICSI outcome and no negative effect. A short incubation time may be beneficial for embryo development and pregnancy outcome; however, excessive incubation (>4 h) should be avoided. However, the incubation time after degranulation remains controversial, and negative effects have been observed upon varying this interval, in addition to studies showing no effect or a favourable outcome. Therefore, whether ICSI should be performed after a period of recovery following degranulation should be investigated further. In conclusion, further multicenter, randomised controlled studies with large sample sizes are warranted to optimise the precise timing of the ICSI procedure in the future.

Abbreviations
ART: Assisted reproductive technology; BDNF: Brain-derived neurotrophic factor; COC: Cumulus-oocyte-complex; DN: Denudation; HCG: Human chorionic gonadotropin; ICSI: Intracytoplasmic sperm injection; MAPK: Mitogen-activated protein kinase; OPU: Oocyte pick-up; ROS: Reactive oxygen species

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Authors’ contributions
Xue Wang and Zheng-Yi Sun contributed to the conception of the study. Xue Wang and Ya-Ling Xiao performed the literature search, data extraction, and study quality assessment. Xue Wang, Jing-Ran Zhen, and Zheng-Yi Sun were involved in statistical analysis. Xue Wang, Ya-Ling Xiao, and Qi Yu contributed to the interpretation of the results. Xue Wang was responsible for manuscript drafting. All authors read and approved the final manuscript.

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Availability of data and materials
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