Early versus late oocyte denudation has no impact on ICSI cycle outcome

Early oocyte denudation does not compromise ICSI cycle outcome: a large retrospective cohort study

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Key message

Oocyte denudation can be carried out at any time up to 5 h following oocyte retrieval without compromising ICSI cycle outcome.

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Abstract

This retrospective cohort study of 2051 consecutive fresh non-donor intracytoplasmic sperm injection (ICSI) cycles investigated whether time from oocyte retrieval to denudation, precisely measured and recorded by an operator-independent automated radiofrequency-based system, affected cycle outcome. ICSI cycles were divided into two groups: group I (denudation within <2 h of oocyte retrieval, n = 1118) and group II (denudation 2–5 h after oocyte retrieval, n = 933). Univariate analysis by two-sample t-test or Mann-Whitney test was used, as appropriate. Both groups were comparable with regards to mean number of oocytes retrieved and fertilized normally after ICSI. The mean number of embryos transferred and surplus embryos cryopreserved at the blastocyst stage were similar. There was no significant difference
in fertilization, embryo implantation, pregnancy, clinical pregnancy or live birth rates between the groups. Analysis of group I ICSI outcome after subdivision into immediate (up to 30 min) and early (31–119 min) denudation showed no statistically significant differences between the two subgroups. In conclusion, early oocyte denudation within <2 h after retrieval does not appear to compromise ICSI cycle outcome, permitting more efficiency and flexibility in scheduling laboratory workload. As this was a retrospective observational study, further prospective studies are required to confirm the findings.

Keywords: fertilization, ICSI, oocyte denudation, oocyte retrieval, pregnancy

Introduction

The use of assisted reproductive technology (ART) has been steadily increasing worldwide (Teoh and Maheshwari, 2014). Between 2010 and 2014, the number of IVF/intracytoplasmic sperm injection (ICSI) cycles performed annually increased by 20% in the UK (HFSA, 2014) and by 27% in the USA (SART, 2014). Approximately two-thirds of those treatment cycles involved the use of ICSI. The increase in demand for ART services has placed significant pressure on IVF laboratories to operate more efficiently in order to manage the expanding workload.

Prior to ICSI, oocyte denudation of the corona–cumulus–complex (CCC) must be performed. The CCC is comprised of cells surrounding the oocyte that undergo orchestrated interactions to allow oocyte pre-ovulatory maturation from the diplotene to the metaphase II stage (MII) (Albertini et al., 2001; Dong et al., 1996). The presence of the CCC has also been suggested to improve oocyte cytoplasmic maturation and metabolism, by stimulating gene expression (McKenzie et al., 2004) and reducing oxidative stress (Fatehi et al., 2005). On the other hand, the cumulus cells block the injecting needle and therefore interfere with the delicate oocyte microinjection. Moreover, because only mature oocytes that have reached the MII stage are suitable for ICSI, optimal optical conditions to allow assessment of the meiotic status of the oocyte are essential, and these become limited in the presence of the cumulus cells (Gardner et al., 2012).

Earlier studies reported that optimization of oocyte nuclear and cytoplasmic maturity requires prolonging the duration of oocyte exposure time to the CCC by pre-incubation prior to denudation post-retrieval. Several studies (Isiklar et al., 2004; Patrat et al., 2012; Rienzi et al., 1998) recommended a post-retrieval window of longer than 2 h prior to oocyte denudation in order to optimize ICSI outcome. Conversely, other studies have reported no influence of the timing of oocyte denudation on ICSI cycle outcome (Bancena et al., 2016; Jacobs et al., 2001; Van de Velde et al., 1998; Yanagida et al., 1998).

In view of the inconsistent and equivocal evidence and conflicting results to date, this study sought to investigate the impact of the interval between oocyte retrieval and denudation on ICSI cycle outcome after accounting for important confounding variables in order to better inform ART laboratory practices.

Materials and methods

Study population

Participants were recruited from a tertiary fertility unit at a London teaching hospital. The study included women who started an ICSI treatment cycle using partner or donor sperm, produced by ejaculation or surgical retrieval where appropriate, for
either male factor or unexplained infertility between 1 January 2015 and 31 October 2016; those who started cycles for the purpose of preimplantation genetic diagnosis or fertility preservation and those requiring oocyte donation were excluded.

**Controlled ovarian stimulation and oocyte retrieval**

FSH injections were started at a dose of 150–450 IU daily for multi-follicular ovarian stimulation in either the mid-luteal long down-regulation or the short gonadotrophin-releasing hormone (GnRH) antagonist protocol as described previously (El-Toukhy et al., 2016; Sunkara et al., 2007). The choice of ovarian stimulation protocol and daily dose of FSH injections was based on female age, ovarian reserve and previous response to ovarian stimulation in earlier cycles. Oocyte maturation was induced using 250 μg of recombinant human chorionic gonadotrophin (HCG) (Ovitrelle; Merck Serono, Darmstadt, Germany) when three or more follicles of 18 mm mean diameter were seen on ultrasound scan. Transvaginal ultrasound-guided retrieval of cumulus–oocyte complexes was performed using the LOGIQ® V1 portable system (GE Healthcare, Milwaukee, WI, USA) strictly 36 h after the HCG injection.

**Embryo transfer and luteal phase support**

Between one and three embryos were transferred under transabdominal ultrasound guidance using the Voluson® 730 system (GE Healthcare) 2, 3 or 5 days after oocyte retrieval, depending on the number and quality of the embryos available.

The Gardner and Schoolcraft blastocyst grading system was adopted (Gardner and Schoolcraft, 1999). According to this classification, a blastocyst of grade 3CC (when the blastocoele completely fills the embryo, with very few cells in both the inner cell mass and the trophectoderm), was considered to be the cut-off for embryo freezing. Surplus blastocysts of grade 3CC or higher that showed no signs of degeneration were cryopreserved on day 5 or 6 after fertilization (Gardner and Schoolcraft, 1999).

The luteal phase was supported with 400 mg progesterone pessaries (Cyclogest®; Actavis UK Ltd, Barnstaple, Devon, UK) twice daily commencing on the day of oocyte retrieval and continuing until 8 weeks’ gestation. A urine pregnancy test using a commercially available kit was performed 16 days after oocyte retrieval to confirm a pregnancy. A clinical pregnancy was defined as the detection of a fetal heartbeat using ultrasound performed 3–4 weeks after a positive pregnancy test. A live birth was defined as delivery of a live baby beyond 24 weeks’ gestation.

**Laboratory procedures**

**Oocyte preparation**

The cumulus–oocyte complexes were identified in sterile plastic dishes (Falcon; Becton Dickinson Labware, Franklin Lakes, NJ, USA), rinsed in holding medium (HM), Quinn’s Advantage Medium with HEPES and 10% (v/v) human serum albumin (Origio, Måløv, Denmark; HM+HSA), and transferred into a four-well Nuncion dish (Thermo Fisher Scientific, Roskilde, Denmark) containing fertilization medium (Quinn’s Advantage) supplemented with 10% (v/v) human albumin solution (Origio, Måløv, Denmark; FM+HSA). The oocytes were incubated at 37°C in an atmosphere of 6% CO₂ and 5% O₂, balanced with nitrogen in a MINC benchtop incubator (Cook Medical, Bloomington, IN, USA), until the time of the denudation procedure. Oocytes were denuded using Cumulase® according to the manufacturer’s instructions (Origio, Måløv, Denmark), assessed for maturity using light microscopy and mature oocytes were incubated in FM+HSA as before until the time of injection. The timing of oocyte
denudation and sperm injection in this study was based solely on workflow and pragmatic considerations.

Sperm preparation

Ejaculated semen samples were collected at the time of oocyte retrieval by masturbation after 2–5 days of abstinence. After evaluation of semen parameters according to World Health Organization criteria (Cooper et al., 2010), samples were separated by buoyant density centrifugation for 20 min at 300g using a discontinuous two-layer gradient (45% and 95% Sil-Select, Ferti Pro NV, Beernem, Belgium). The pellet was collected and re-suspended in 6 ml FM+HSA and centrifuged for 10 min at 300g. The final pellet was re-suspended in 1 ml FM+HSA and incubated at 37°C in an atmosphere of 6% CO₂ in air until the time of ICSI.

ICSI procedure

All mature oocytes (MII stage) were injected with sperm, with ICSI (Abdelmassih et al., 1996; Nagy et al., 1995; Palermo et al., 1992; Van Steirteghem et al., 1995) carried out on the heated stage of an inverted-phase microscope (Diaphot, Nikon Corporation, Tokyo, Japan) at 400× magnification, using the Hoffman modulation contrast system (Modulation Optics Inc., Greenvale, NY, USA). During ICSI, sperm were selected and immobilized in PVP 7% solution (Origio, Måløv, Denmark), oocytes were placed in HM+HSA and secured using a holding pipette with the polar body at the 6 or 12 o’clock position and the injection pipette was inserted at the 3 o’clock position (Nagy et al., 1995). After ICSI, oocytes were rinsed through three 40 µl droplets of FM+HSA before overnight group culture incubation in a single 40 µl drop of FM+HSA under oil for tissue culture (Origio, Måløv, Denmark) in the benchtop incubator as before.

Timing record

The precise timing of all laboratory procedures was recorded by an operator-independent automated radiofrequency-based system (RI-Witness™, Research Instruments, Falmouth, Cornwall, UK). The interval between oocyte retrieval and denudation was calculated as that between the time of commencement of oocyte retrieval and the time of the beginning of denudation. The interval between completion of oocyte denudation and commencement of ICSI was also recorded.

Fertilization check

Oocytes were assessed for fertilization 18–20 h after ICSI, with normal fertilization indicated by the presence of two-pronuclear (2PN) within the oocyte. Normally fertilized oocytes were transferred and cultured as before in 40 µl drops of SAGE 1-Step (Origio, Måløv, Denmark) overlaid with mineral oil. The day of embryo transfer (2, 3 or 5) was assigned according to the number of 2PN embryos and patient age according to the protocol of the Unit.

Embryoe assessment

Cleavage-stage embryos were assessed on day 2 and/or day 3 after oocyte retrieval and scored according to the number and symmetry of blastomeres and degree of fragmentation (Bolton et al., 1989). For patients having embryo transfer on day 5 after oocyte retrieval, embryos were assessed on the morning of day 5 and graded according to blastocoele expansion, and the quality of the trophoectoderm and inner cell mass cells (Gardner and Schoolcraft, 1999). For all cycles, surplus blastocysts of
grade 3CC or higher that showed no signs of degeneration were cryopreserved on day 5/6.

Data collection and statistical analysis

Because the present retrospective study did not involve either therapeutic interventions or change to routine ICSI protocols and used routinely collected ICSI cycle data, the study did not require additional approval from the institutional ethics committee. However, each couple gave written informed consent for the use of the cycle data anonymously upon entering our IVF programme and before starting an ICSI cycle, according to HFEA stipulation.

Data were collected for female patient demographics, ICSI cycle characteristics, oocyte retrieval outcome, semen parameters, and the number of embryos that were considered suitable for transfer or freezing. ICSI cycles were divided into two groups based on whether the retrieval-denudation time was less than 2 h (group I) or 2 h and longer (group II) (Isiklar et al., 2004; Patrat et al., 2012; Rienzi et al., 1998). Group I was further subdivided into immediate denudation (within 30 min) and early denudation (31–119 min).

The study outcome measures were oocyte fertilization, embryo implantation, pregnancy, clinical pregnancy and live birth rates. Univariate analysis of the study outcome measures and the associated clinical variables was performed using two-sample t-test or Mann-Whitney test, as appropriate. A multivariate logistic regression analysis was used to adjust for important confounding variables, including female age, ICSI attempt number, duration of ovarian stimulation, number of oocytes retrieved, semen status, duration between oocyte denudation and sperm injection, number of embryos transferred and surplus embryos available for cryopreservation. StatView software (StatView Corporation, Berkeley, CA, USA) was used for statistical analysis. A P-value of <0.05 was considered as statistically significant.

Results

Between 1st January 2015 and 31st October 2016, data for a consecutive series of 2051 fresh non-donor ICSI cycles were collected and included in the study. The mean age of women in the study was 35.6 ± 4.4 years, with 62% aged 35 years and older. Fresh sperm was used for ICSI in 88% of cycles. The mean (± SD) number of oocytes retrieved, injected with sperm and fertilized normally per cycle was 11.2 ± 6.7, 9.0 ± 5.6 and 6.2 ± 4.4, respectively. The fertilization rate per injected oocyte was 68%. Overall, 97% of ICSI cycles resulted in embryo transfer and the mean number of embryos transferred per cycle was 1.6 ± 0.6. In 42% of cycles, surplus embryos of suitable quality for cryopreservation developed, with a mean 3.0 ± 1.2 embryos cryopreserved per cycle. The implantation, pregnancy, clinical pregnancy and live birth rates per cycle were 27%, 38%, 31% and 25%, respectively.

The interval between oocyte retrieval and denudation (retrieval-denudation time) varied between 12 min and 5 h (mean 120 ± 56 min). The mean retrieval-denudation time was similar in cycles where a biochemical pregnancy was achieved and in unsuccessful cycles (121 ± 59 min versus 119 ± 55 min, respectively). Likewise, the mean interval between oocyte denudation and ICSI was similar in cycles that did and those that did not result in pregnancy (111 ± 72 versus 112 ± 69 min, respectively).

There was no statistically significant difference in the pregnancy, clinical pregnancy and live birth rates in relation to the retrieval-denudation time (Figure 1). The likelihood of a live birth was not influenced by the retrieval-denudation time (in
minutes), after controlling for important confounding variables, including female age, ICSI attempt number, duration of ovarian stimulation (in days), number of oocytes retrieved, semen status (ejaculated versus surgically retrieved and fresh versus frozen), duration between oocyte denudation and sperm injection (in minutes), number of embryos transferred and achieving freezing of surplus embryos (adjusted odds ratio [OR] = 1.0, 95% confidence [CI], 0.99–1.002). Likewise, the duration between oocyte denudation and sperm injection (in minutes) did not influence the likelihood of a live birth after adjusting for the same variables above (adjusted OR = 1.0, 95% CI, 0.99–1.001).

In order to account for important confounding variables, the ICSI cycles included in the study were separated into two groups: early denudation (retrieval-denudation time up to 1 h and 59 min, n = 1118) and delayed denudation (retrieval-denudation time of 2–5 h, n = 933). The two groups were not significantly different in terms of patient demographics and ovarian stimulation characteristics including female age, ICSI attempt number studied, ovarian stimulation regimen, duration of FSH stimulation, total FSH dose and parameters of semen used for ICSI (Table 1).

The two groups were also comparable in relation to the number of oocytes retrieved, injected with sperm and that fertilized normally. There were no significant differences between the two groups in the mean number of embryos transferred, day of embryo transfer, proportion of cycles where supernumerary embryos were cryopreserved, and the mean number of embryos cryopreserved. The oocyte fertilization, embryo implantation, pregnancy, clinical pregnancy and live birth rates were also similar between the two groups (Table 2).

After controlling for the same important confounding variables mentioned earlier, the likelihood of a live birth in cycles in which the retrieval-denudation time was shorter than 2 h was not significantly different from that in cycles in which the retrieval-denudation time was 2 h or longer (adjusted OR = 1.13, 95% CI, 0.91–1.39).

The ICSI cycles in which the retrieval-denudation time was shorter than 2 h were further subdivided into two subgroups: immediate denudation (retrieval-denudation time of up to 30 min, n = 66) and early denudation (retrieval-denudation time of 31–119 min, n = 1052). The two groups were not significantly different in terms of patient demographics, ovarian stimulation characteristics and ICSI cycle outcome (Table 3). Likewise, there was no significant difference in any of those variables between the immediate denudation subgroup (retrieval-denudation time of up to 30 min, n = 66) and the delayed denudation group (retrieval-denudation time of 2–5 h, n = 933) (data not shown).

**Discussion**

To date, this is the largest study to examine the impact of different retrieval-denudation times on fresh non-donor ICSI cycles. The study used an operator-independent automated system for recording the retrieval-denudation time, and the results showed that ICSI outcome is not influenced by a retrieval-denudation time ranging between 1 h and 5 h. Early oocyte denudation (within <2 h of retrieval) was associated with similar fertilization, implantation and pregnancy rates compared with delayed oocyte denudation (2–5 h after retrieval). Likewise, performing oocyte denudation within the first 30 min or 31–119 min of retrieval did not influence ICSI outcome. Markers of embryo developmental potential, including surplus blastocysts available for cryopreservation and embryo implantation rates, were also comparable between early and delayed denudation. These results are in accordance with earlier
but smaller studies, which reported that delaying oocyte denudation for ICSI did not improve cycle outcomes (Jacobs et al., 2001; Van de Velde et al., 1998; Yanagida et al., 2004).

Our results are also consistent with those of a recent large study involving nearly 4000 ICSI cycles (Barcena et al., 2016), which showed no significant relationship between different oocyte retrieval to denudation times and ICSI outcome. The study by Barcena et al. (2016) exclusively included young oocyte donors (mean age 26.9 years) and a mixture of fresh and vitrified/warmed oocytes. The study also used GnRH agonist for triggering oocyte maturation, mostly used frozen sperm for ICSI and performed cleavage-stage embryo transfer (Barcena et al., 2016). Together, the current study and that by Barcena et al. (2016) indicate that the retrieval-denudation time does not significantly affect ICSI cycle outcome regardless of female age, cause of infertility (i.e. female, male or unexplained), whether own or donated oocytes were inseminated, triggering oocyte maturation was induced by HCG or GnRH agonist, fresh or frozen sperm was used for ICSI or whether cleavage-stage embryos or blastocysts were transferred, thus allowing the results to be more generalized and applicable to a wider scope of clinical practice.

The present study contrasts with previous reports (Dozortsev et al., 2004; Ho et al., 2003; Isiklar et al., 2004, Patrat et al., 2012; Rienzi et al., 1998), which suggested that a delay of more than 2 h before oocyte denudation could improve oocyte maturity, fertilization rate, embryo quality or ICSI outcome. However, most of those studies included a small sample size and recorded the steps of the ICSI procedure timings manually. These studies also reported conflicting results in terms of specific outcome measures and did not account for important confounding variables including patient demographics, ICSI cycle characteristics and sperm parameters.

The strengths of this study are the large number of ICSI cycles studied, permitting valid comparisons of cycle outcomes within the study groups, recording of the oocyte retrieval-denudation time using an operator-independent automated radiofrequency-based system, reporting of live birth outcome and accounting for important confounding variables. Nevertheless, it is important to highlight that this study was retrospective and therefore the possibility of bias due to variables not included in the analysis cannot be excluded. In addition, the results do not indicate whether an oocyte retrieval-denudation time of longer than 5 h could influence ICSI outcome. It has been suggested that a prolonged oocyte retrieval to denudation delay of up to 6 h could have a positive effect on ICSI outcomes (Falcone et al., 2008), whereas a delay of 11 h or longer between oocyte retrieval and completing ICSI may have a detrimental impact on cycle outcome (Tesarik et al., 1994; Yanagida et al., 1998). It is also important to note that although this study suggested that the denudation to ICSI time did not influence cycle outcome, this variable will need to be studied separately in future studies to confirm these findings.

The rapid expansion in the use of fertility services has placed an ever-increasing demand on ART laboratories to schedule their workflow more efficiently and maximize workload output (Teoh and Maheshwari, 2014). The results of this study indicate that the insemination window is wider than previously suggested (Dozortsev et al., 2004). A delay of over 2 h between oocyte retrieval and starting the ICSI procedure could represent a significant challenge for the efficient scheduling of clinical and laboratory procedures, particularly when oocyte retrievals are carried out in the afternoon in busy IVF clinics. Flexibility in the window during which an ICSI procedure can be completed without compromising cycle outcome could facilitate extending the period during which oocyte retrievals could be scheduled during weekdays and on weekends,
hence avoiding significant prolongation of the working hours of laboratory practitioners. Improving laboratory scheduling efficiency could also help to reduce the overall cost of the ICSI cycle (Teoh and Maheshwari, 2014), thus improving IVF and ICSI accessibility.

In conclusion, this study suggests that oocyte denudation within 2 h of retrieval is associated with a comparable ICSI cycle outcome compared with oocyte denudation between 2 and 5 h after retrieval. Further prospective and preferably randomized studies are needed to confirm this result.

References


Fatehi AN, Roelen BA, Colenbrander B, Schoevers EJ, Gadella BM, Beverst MM, van den Hurk R. Presence of cumulus cells during in vitro fertilization protects the bovine oocyte against oxidative stress and improves first cleavage but does not affect further development. Zygote. 2005 May; 13(2): 177–85


Declaration: The authors report no financial or commercial conflicts of interest.

Figure 1.

Comment [SH1]: AUTHOR: Please provide caption for Figure 1.
Table 1– Patient and ICSI cycle characteristics for women in groups I (retrieval-denudation time up to 1 h and 59 min) and II (retrieval-denudation time of 2–5 h).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group I (n = 1118)</th>
<th>Group II (n = 933)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years (%)</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>35–39 years (%)</td>
<td>41</td>
<td>44</td>
</tr>
<tr>
<td>40–42 years (%)</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>43–45 years (%)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Mean ICSI attempt number (%)</td>
<td>1.5 (1.0)</td>
<td>1.6 (1.0)</td>
</tr>
<tr>
<td>Sperm status (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Frozen</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Ejaculated</td>
<td>95</td>
<td>96</td>
</tr>
<tr>
<td>Surgically retrieved</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Mean post-preparation sperm motility (%)</td>
<td>89 (20)</td>
<td>88 (20)</td>
</tr>
<tr>
<td>Mean motile sperm count in final preparation (million/ml)</td>
<td>7.4 (10.4)</td>
<td>7.0 (10.5)</td>
</tr>
<tr>
<td>Ovarian stimulation protocols (%) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short antagonist</td>
<td>72</td>
<td>68</td>
</tr>
<tr>
<td>Long agonist</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>Total FSH dose (IU)</td>
<td>2473 (1229)</td>
<td>2458 (1204)</td>
</tr>
<tr>
<td>Duration of ovarian stimulation (days)</td>
<td>11.1 (2.1)</td>
<td>11.0 (2.1)</td>
</tr>
</tbody>
</table>

There were no statistically significant differences between the two groups. Data are provided as mean (SD) or as shown.

ICSI = intracytoplasmic sperm injection.
Table 2 – ICSI cycle outcomes for study groups I (retrieval-denudation time up to 1 h and 59 min) and II (retrieval-denudation time of 2–5 h).

| Outcomes                                | Group I  
|                                         |  
| (n = 1118)                              |  
| Mean retrieval-denudation time, minutes | 79 (59)  
| Mean number of oocytes retrieved        | 11.2 (6.7)  
| Mean number of MII oocytes injected     | 9.0 (5.7)  
| Mean number of fertilized oocytes       | 6.2 (4.4)  
| Fertilization rate/oocyte retrieved (%) | 55  
| Fertilization rate/oocyte injected (%)  | 68  
| Number of cycles reaching ET (%)        | 1083 (97)  
| Day 2 embryo                            | 125 (12)  
| Day 3 embryo                            | 258 (24)  
| Day 5 embryo                            | 700 (65)  
| Mean number of embryos transferred/ET   | 1.6 (0.64)  
| One embryo transferred (n, %)           | 485 (45)  
| Two embryos transferred (n, %)          | 501 (46)  
| Three embryos transferred (n, %)        | 97 (9)  
| Number of cycles with surplus embryos   | 466 (42)  
| cryopreserved (%)                        | 3 (2.3)  
| Mean number of embryos cryopreserved     | 2.8 (2.2)  
| Implantation rate (%)                   | 27 (481/1781)  
| Pregnancy rate (%)                      | 38  
| Clinical pregnancy rate (%)             | 32  
| Live birth rate (%)                     | 26  

Data are provided as mean (SD) or as shown.

ET = embryo transfer; ICSI = intracytoplasmic sperm injection; MII = metaphase II;

\(^a\) P < 0.0001, no other statistically significant differences between the two groups.
Table 3 – ICSI cycle outcomes in group I based on the oocyte retrieval-denudation time.

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Denudation within 30 min (n = 66)</th>
<th>Denudation within 31–119 min (n = 1052)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean time, minutes*</td>
<td>24 (6)</td>
<td>82 (21)</td>
</tr>
<tr>
<td>Mean age in years</td>
<td>35.5 (4.9)</td>
<td>35.5 (4.5)</td>
</tr>
<tr>
<td>Mean ICSI cycle order</td>
<td>1.5 (0.68)</td>
<td>1.6 (0.99)</td>
</tr>
<tr>
<td>Sperm status (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>85</td>
<td>88</td>
</tr>
<tr>
<td>Frozen</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Ejaculated</td>
<td>94</td>
<td>95</td>
</tr>
<tr>
<td>Surgically retrieved</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Duration of ovarian stimulation (days)</td>
<td>11.1 (2.1)</td>
<td>11.1 (2.1)</td>
</tr>
<tr>
<td>Mean number of oocytes retrieved</td>
<td>11.8 (7.6)</td>
<td>11.2 (6.7)</td>
</tr>
<tr>
<td>Mean number of MII oocytes injected</td>
<td>9.8 (6.2)</td>
<td>9.0 (5.7)</td>
</tr>
<tr>
<td>Mean number of fertilized oocytes</td>
<td>6.7 (5.0)</td>
<td>6.2 (4.4)</td>
</tr>
<tr>
<td>Fertilization rate/oocyte retrieved (%)</td>
<td>57</td>
<td>55</td>
</tr>
<tr>
<td>Fertilization rate/oocyte injected (%)</td>
<td>67</td>
<td>68</td>
</tr>
<tr>
<td>Number of cycles reaching ET (%)</td>
<td>63 (95)</td>
<td>1019 (97)</td>
</tr>
<tr>
<td>Mean number of embryos transferred/ET</td>
<td>1.5 (0.64)</td>
<td>1.6 (0.64)</td>
</tr>
<tr>
<td>Mean number of embryos cryopreserved</td>
<td>2.9 (1.6)</td>
<td>3.0 (2.4)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>29 (29/100)</td>
<td>27 (452/1681)</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>35</td>
<td>38</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>35</td>
<td>32</td>
</tr>
<tr>
<td>Live birth rate (%)</td>
<td>27</td>
<td>26</td>
</tr>
</tbody>
</table>

Data are provided as mean (SD) or as shown.

*P < 0.0001, no other statistically significant differences between the two groups.
Figure 1 O Naji RBMO V3.png