Archives of Gynecology and Obstetrics
https://doi.org/10.1007/s00404-020-05555-w

GYNECOLOGIC ENDOCRINOLOGY AND REPRODUCTIVE MEDICINE

Does timing in ICSI cycle affect oocyte quality and reproductive outcomes? A prospective study

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Received: 3 December 2019 / Accepted: 20 April 2020

Abstract

Purpose To evaluate the association of time intervals between various steps of the intracytoplasmic sperm injection (ICSI) cycle with oocyte quality and reproductive outcomes.

Methods We conducted a prospective study among patients undergoing ICSI cycles in an academic hospital between May 2017 and January 2019. The time intervals between the various steps of cycles were recorded. The ICSI cycles were categorized according to the different time intervals; human chorionic gonadotropin (hCG) injection to oocyte pick up (hCG-OPU) (≤ 36 h and > 36 h), OPU-denudation (≤ 2 h and > 2 h), and denudation-ICSI (≤ 2 h and > 2 h). The main outcome measures were oocyte dysmorphisms, fertilization, cleavage, biochemical, and clinical pregnancy rates.

Results A total of 613 ICSI cycles using fresh autologous oocytes were included in this study. After adjusting for confounders, the hCG–OPU interval was associated with the presence of cytoplasmic granulation, inclusion body, and also the total number of morphologically abnormal premature oocytes in the cycle ($P = 0.02$, $P = 0.04$, $P = 0.008$, respectively). OPU-denudation interval was associated with cytoplasmic granulation and extended perivitelline space of the oocytes ($P = 0.006$ and $P = 0.03$, respectively). The denudation-ICSI interval was only associated with cytoplasmic granulation ($P = 0.01$). However, hCG–OPU, OPU–denudation, and denudation–ICSI intervals were not significantly associated with fertilization, cleavage, biochemical, and clinical pregnancy rates.

Conclusions All the studied time intervals between various steps of ICSI procedure could affect oocyte quality, but the oocyte dysmorphisms were mainly associated with hCG-OPU interval. However, the time intervals were not associated with fertilization, cleavage, and pregnancy outcomes.

Keywords Intracytoplasmic sperm injection · Timing · Oocyte dysmorphisms · Fertilization · Pregnancy

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Published online: 04 May 2020

Introduction

Intracytoplasmic sperm injection (ICSI) is a technique in assisted reproductive technology (ART) for the treatment of infertile couples in which a sperm is inserted into the cytoplasm of the oocyte. Oocyte quality is a decisive factor for fertilization and embryo development [1, 2]. In vivo maturation of oocytes before aspiration from ovaries may affect oocyte quality, and subsequent fertilization and embryo development [3]. Moreover, following oocytes aspiration from ovaries, due to the possibility of gene expression alteration and reduction of spindle stability, the fertilization potential of oocytes is progressively declined with prolonged staying in in vitro condition [4]. Impaired quality of oocytes may lead to poor developmental competence of derived embryos. Regarding the limited fertilizable lifespan of oocytes, they should be fertilized before the reduction in the quality and fertilization potential to yield viable and high-quality embryos [5]. Hence, the exposure time of the oocytes to the both in vivo and in vitro environments before fertilization may have crucial importance to obtain high-quality oocytes and embryos, and therefore the reproductive outcomes of ICSI cycles may be affected by the ART laboratory timing.

Based on the daily workload of ART laboratories, the intervals between steps of ICSI cycles vary considerably in the daily operation of ART laboratories, and there is no standard for the duration of intervals between different steps of ICSI cycles (human chorionic gonadotrophin [hCG] administration, oocyte pick up [OPU], denudation, and ICSI) in ART laboratories. So, there is still clinical concern about the impact of these time intervals on the outcomes of ICSI cycles.

Although it seems that the timing in ICSI cycles may have effects on the outcomes of the procedure, there is a lack of sound evidence regarding the issue of timing on the oocyte quality, developmental competence of embryos, and clinical outcomes. Moreover, the current findings in this area are contradicting and derived from reports with retrospective design [5–12]. Therefore, in the current study, we aimed to assess the effect of in vivo time interval between hCG administration to OPU, and also in vitro time intervals (OPU to denudation, and denudation to ICSI) on the oocyte quality and outcomes of ICSI cycles.

Material and methods

Study population and ethical approval

This is a prospective study of 613 ICSI cycles conducted between May 2017 and January 2019 in the IVF center of Taleghani hospital in Tehran. We included fresh ICSI cycles, and exclusion criteria were cycles with in-vitro maturation, oocyte donation, activation of the oocyte, preimplantation genetic diagnosis, testicular biopsies, frozen sperm or oocyte, and patients with cancer or hepatitis. All participants gave written informed consent before entering the study. The ethics committee of Shahid Beheshti University of Medical Sciences approved this study (IR.SBMU.RETECH.REC.1397.510).

Ovarian stimulation and oocyte pick-up

Ovarian stimulation was accomplished either with Gonadotropin-Releasing Hormone (GnRH) antagonist or agonist protocols, based on individual patients' parameters. The stimulation procedure was initiated using recombinant FSH (Gonal-F, Merck Serono Europe Ltd, UK; or Puregon, MSD, New Jersey, USA), and patients were monitored by serial ultrasonography until at least three follicles (> 17 mm diameter) were visualized in the ovaries. Ovulation was triggered by the injection of hCG (Pregnyl; MSD, Brussels, Belgium) at 9:00 pm. OPU was accomplished 35–39 h after hCG administration (two cases of patients had the hCG-OPU time of 39.3 h, one case with a time of 40.3 h, and another one with a record of 41.7 h) by transvaginal aspiration under ultrasound guidance, and subsequently denudation was performed for removing cumulus cells.

ICSI and embryo transfer

Semen samples were collected by masturbation and processed according to the World Health Organization (WHO) guidelines at the time of OPU [13]. Just before performing ICSI, all mature denuded oocytes were morphologically assessed according to the guidelines of the European Society of Human Reproduction and Embryology [14]. The presence of intracytoplasmic (granulation, vacuole, smooth endoplasmic reticulum [SER] clustering, and inclusion body in the cytoplasm of oocytes) and extracytoplasmic dysmorphisms (shape of oocyte, perivitelline space [PVS], polar body [PB], zona pellucida [ZP]) were recorded. Afterward, all mature oocytes were inseminated by ICSI. Fertilization was confirmed by the presence of two pronuclei between 18 and 20 h after ICSI. The embryos were cultured in Global medium (LifeGlobal® ART media) and transferred to the

uterus on day 3. Biochemical pregnancy was assessed by serum beta-HCG level, 14 days after embryo transfer, and clinical pregnancy was determined by ultrasound visualization of the gestational sac at about weeks 5–6 of gestation.

Time recording

No intervention was applied in the studied ICSI cycles, and all the procedures were performed according to our routine clinical management. The diversity in the time intervals between different steps of ICSI cycles was due to the difference in the daily workload of our unit. We accurately recorded the precise time of fulfilling different steps of the ICSI cycle: OPU, denudation, and ICSI.

Data analysis

The time intervals between hCG–OPU, OPU–denudation, and denudation–ICSI were recorded, and statistical analysis was performed using SPSS software 22 (SPSS Inc., Chicago, IL). We presented data as mean ± standard deviation (SD) for continuous variables and frequency (percentage) for categorical variables. Kruskal–Wallis (with Dunn's multiple comparisons test) and χ^2 test were utilized for analysis of continuous and categorical variables, respectively. The main outcome measures of this work were oocyte dysmorphisms, rates of fertilization, cleavage, biochemical pregnancy, and clinical pregnancy. To adjust the effects of confounding variables, multivariate Poisson, linear, and logistic regressions were performed for countable (oocyte dysmorphisms), continuous (fertilization and cleavage rate), and dichotomous (biochemical pregnancy and clinical pregnancy) outcomes, respectively. *P*-value of < 0.05 was considered as statistically significant.

Results

In this study, 613 ICSI cycles with a total number of 5057 aspirated oocytes were included, and time intervals for the various steps of cycles were recorded. We categorized ICSI cycles based on the different time intervals and appraised the oocyte quality and reproductive outcomes of the cycles. The cycles were initially categorized according to the hCG-OPU intervals (≤ 36 h and > 36 h) and then based on the OPU–denudation intervals (≤ 2 h and > 2 h), and subsequently, each of the latter groups was divided according to denudation–ICSI intervals into two subgroups (≤ 2 h and > 2 h). Demography and characteristics of ICSI cycles in the studied groups were shown in Table 1. There were no significant differences among the basic characteristics of studied groups except for the female age ($P = 0.003$) and decreased ovarian reserve ($P = 0.004$) (Table 1).

Total aspirated and metaphase 2 (MII) oocytes were significantly differed ($P = 0.0006$ and $P = 0.004$, respectively) among different studied groups (Table 2). Moreover, we did not notice any significant difference regarding to the ratio of MII pre total aspirated oocytes and different oocyte dysmorphisms (the presence of cytoplasmic granulation, central granulation, vacuole, SER clustering, and inclusion body in oocytes, amorph, oval-shaped, or dark oocyte, extended or granulated PVS, multiple fragmented PB, thin or thick ZP, and double fragmented PB) between studied groups, except for huge PB oocytes ($P = 0.002$) (Table 2). Also, the average oocyte quality index (AOQI, the total number of dysmorphisms for all metaphase II oocytes in a cycle divided by the number of metaphase II oocytes in a cycle) did not show any significant change in different time intervals (Table 2).

No significant changes in fertilization, cleavage, biochemical, and clinical pregnancy rates were observed in different hCG–OPU, OPU–denudation, and denudation–ICSI intervals (Table 3).

Multivariable analysis was conducted to adjust outcome measures of this study for confounding variables (female age, cause of infertility [decreased ovarian reserve, recurrent implantation failure, and male infertility], total aspirated oocytes, total dose of gonadotrophin, body mass index, anti mullerian hormone level, and primary or secondary infertility) (Table 4). We found that the hCG–OPU interval was significantly associated with the presence of cytoplasmic granulation, inclusion body in the cytoplasm of oocyte, and AOQI ($P = 0.02$, $P = 0.04$, $P = 0.008$, respectively) (Table 4). The OPU-denudation interval was associated with cytoplasmic granulation and extended PVS of oocytes ($P = 0.006$ and $P = 0.03$, respectively) (Table 4). The denudation-ICSI interval was associated with cytoplasmic granulation of oocytes ($P = 0.01$) (Table 4). Nevertheless, fertilization, cleavage, biochemical, and clinical pregnancy rates were not associated with the time intervals in ICSI cycles, even after adjusting confounders to outcomes (Table 4).

Discussion

We conducted the first prospective study, to the best of our knowledge, evaluated the importance of timing of various steps of the ICSI cycle in the reproductive outcomes and oocyte quality. ICSI outcomes were analyzed in groups which classified according to the different hCG–OPU, OPU–denudation, and denudation–ICSI intervals. The results of the current study revealed no significant association between different time intervals and the fertilization, cleavage, biochemical, and clinical pregnancy rates. However, there were significant associations between time intervals in the ICSI cycle and some specific oocyte dysmorphisms.

Table 1 Demographic and ICSI cycle characteristics of patients

Variable	HCG–OPU ≤ 36 h ($n=264$)				HCG–OPU > 36 h ($n=349$)				*P*-value
	OPU–DN ≤ 2 h ($n=141$)		OPU–DN > 2 h ($n=123$)		OPU–DN ≤ 2 h ($n=180$)		OPU–DN > 2 h ($n=169$)		
	DN–ICSI ≤ 2 h ($n=101$)	DN–ICSI > 2 h ($n=40$)	DN–ICSI ≤ 2 h ($n=75$)	DN–ICSI > 2 h ($n=48$)	DN–ICSI ≤ 2 h ($n=119$)	DN–ICSI > 2 h ($n=61$)	DN–ICSI ≤ 2 h ($n=104$)	DN–ICSI > 2 h ($n=65$)	
hCG–OPU interval (h), mean ± SD, range	35.7 ± 0.3, 35.0–36.0	35.8 ± 0.2, 35.0–36.0	35.7 ± 0.3, 35.0–36.0	35.7 ± 0.2, 35.1–36.0	36.8 ± 0.8, 36.1–41.7	36.7 ± 0.5, 36.1–38.2	36.9 ± 0.8, 36.1–40.3	36.7 ± 0.5, 36.1–38.7	
OPU–denudation (h), mean ± SD, range	1.5 ± 0.4, 0.5–2.0	1.4 ± 0.4, 0.6–2.0	3.2 ± 1.1, 2.1–5.8	2.9 ± 0.9, 2.1–6.0	1.3 ± 0.5, 0.2–2.0	1.4 ± 0.4, 0.2–2.0	3.4 ± 1.1, 2.1–6.0	3.2 ± 0.8, 2.1–5.5	
Denudation–ICSI (h), mean ± SD, range	1.1 ± 0.5, 0.1–2.0	3.3 ± 1.0, 2.2–5.8	1.0 ± 0.5, 0.1–2.0	3.6 ± 1.2, 2.3–6.0	1.2 ± 0.6, 0.1–2.0	3.4 ± 1.1, 2.1–6.0	1.1 ± 0.5, 0.1–2.0	3.0 ± 0.8, 2.1–4.8	
Female age (years)	33.6 ± 6.1	33.6 ± 6.5	31.2 ± 6.0[a]	32.7 ± 5.8	34.5 ± 6.5[ab]	34.4 ± 5.9	31.8 ± 5.8[b]	32.3 ± 5.5	0.003
BMI (kg/m^2)	26.0 ± 4.1	25.7 ± 3.8	26.0 ± 3.3	26.2 ± 3.9	25.4 ± 4.1	24.5 ± 3.4	25.0 ± 3.9	26.7 ± 3.6	0.02
FSH (IU/mL)	6.5 ± 4.2	5.9 ± 2.9	5.8 ± 2.3	6.7 ± 4.8	6.1 ± 4.6	5.1 ± 2.5	6.3 ± 4.2	7.1 ± 7.9	0.9
AMH (ng/mL)	3.2 ± 2.9	3.8 ± 3.4	3.8 ± 3.9	3.8 ± 3.9	3.4 ± 3.4	4.7 ± 4.7	3.7 ± 4.1	4.5 ± 4.0	0.3
Primary infertility (*n*)	74 (73.3)	36 (90.0)	62 (82.7)	42 (87.5)	90 (75.6)	49 (80.3)	88 (84.6)	56 (86.1)	0.1
Secondary infertility (*n*)	27 (26.7)	4 (10.0)	13 (17.3)	6 (12.5)	29 (24.4)	12 (19.7)	16 (15.4)	9 (13.9)	
Infertility duration (years)	5.3 ± 4.6	7.2 ± 6.7	4.8 ± 3.7	4.6 ± 3.6	5.6 ± 4.7	5.9 ± 4.7	5.3 ± 4.8	5.2 ± 4.3	0.6
No. of previous ICSI cycles									
0	64 (63.4)	28 (70.0)	52 (69.3)	30 (62.5)	74 (62.1)	33 (54.1)	75 (72.1)	45 (69.2)	0.4
1	27 (26.7)	8 (20.0)	14 (18.7)	13 (27.1)	27 (22.7)	13 (21.3)	17 (16.3)	10 (15.4)	
2	8 (7.9)	2 (5.0)	7 (9.3)	5 (10.4)	14 (11.8)	10 (16.4)	6 (5.8)	6 (9.2)	
≥ 3	2 (2.0)	2 (5.0)	2 (2.7)	0 (0)	4 (3.4)	5 (8.2)	6 (5.8)	4 (6.2)	
Cause of infertility (*n*)									
Male factor	47 (46.5)	24 (60.0)	39 (52.0)	21 (43.7)	41 (34.4)	27 (44.3)	49 (47.1)	29 (44.6)	0.1
AMA	12 (11.9)	2 (5.0)	3 (4.0)	6 (12.5)	13 (10.9)	7 (11.5)	6 (5.8)	7 (10.8)	0.4
DOR	21 (20.8)	7 (17.5)	6 (8.0)	6 (12.5)	26 (21.8)	6 (9.8)	8 (7.7)	4 (6.1)	0.004
PCOS	25 (24.7)	11 (27.5)	16 (21.3)	16 (33.3)	20 (16.8)	14 (22.9)	22 (21.1)	18 (27.7)	0.4
Endometriosis	4 (4.0)	1 (2.5)	3 (4.0)	2 (4.2)	8 (6.7)	0 (0)	4 (3.8)	0 (0)	0.3
RIF	7 (6.9)	4 (10.0)	6 (8.0)	2 (4.2)	7 (5.9)	10 (16.4)	6 (5.8)	9 (13.8)	0.1
Uterine	7 (6.9)	1 (2.5)	5 (6.7)	2 (4.2)	12 (10.1)	4 (6.6)	6 (5.8)	6 (9.2)	0.7
Others	14 (13.9)	5 (12.5)	10 (13.3)	5 (10.4)	17 (14.3)	9 (14.7)	18 (17.3)	10 (15.4)	0.9
Stimulation protocol (*n*)									
Antagonist	97 (96.0)	37 (92.5)	71 (94.7)	45 (93.7)	110 (92.4)	56 (91.8)	97 (93.3)	63 (96.9)	0.8
Agonist	4 (4.0)	3 (7.5)	4 (5.3)	3 (6.3)	9 (7.6)	5 (8.2)	7 (6.7)	2 (3.1)	
Total dose of gonadotrophins (IU)	2406.0 ± 1384.3	2815.6 ± 1435.2	2234.7 ± 956.7	1909.1 ± 994.1	2636.7 ± 1310.7	2581.4 ± 1548.7	2526.8 ± 1328.6	2076.9 ± 908.0	0.09
No. of embryos transferred	1.7 ± 0.5	1.7 ± 0.6	1.8 ± 0.5	1.7 ± 0.4	1.8 ± 0.6	1.9 ± 0.5	1.8 ± 0.5	1.7 ± 0.5	0.7

Data were presented as mean ± SD, number (percentage), or as shown. Comparisons with the identical superscripts reveal significant differences (Regarding the Female age variable, the adjusted *P*-value of post hoc tests were as follow; a: *P*-value = 0.014, b: *P*-value = 0.017, the others are > 0.05)

HCG-OPU hCG injection to oocyte pick up interval; *OPU-DN* oocyte pick up to denudation interval; *DN-ICSI* denudation to ICSI interval; *ICSI* intracytoplasmic sperm injection; *hCG* human chorionic gonadotrophin; *OPU* oocyte pick up; *AMA* advanced maternal age; *DOR* decreased ovarian reserve; *PCO* polycystic ovary; *RIF* recurrent implantation failure; *Others* endocrinology disorders, recurrent pregnancy loss, tubal factor, and unexplained infertility; *BMI* body mass index; *FSH* follicle stimulating hormone; *AMH* anti-mullerian hormone

Table 2 Characteristics of oocytes in ICSI cycles

Variable	HCG–OPU ≤ 36 h ($n=264$)				HCG–OPU > 36 h ($n=349$)				*P*-value
	OPU–DN ≤ 2 h ($n=141$)		OPU–DN > 2 h ($n=123$)		OPU–DN ≤ 2 h ($n=180$)		OPU–DN > 2 h ($n=169$)		
	DN–ICSI ≤ 2 h ($n=101$)	DN–ICSI > 2 h ($n=40$)	DN–ICSI ≤ 2 h ($n=75$)	DN–ICSI > 2 h ($n=48$)	DN–ICSI ≤ 2 h ($n=119$)	DN–ICSI > 2 h ($n=61$)	DN–ICSI ≤ 2 h ($n=104$)	DN–ICSI > 2 h ($n=65$)	
Total number of oocytes aspirated (mean ± SD)	717 (7.1 ± 4.5)	330 (8.2 ± 5.3)	654 (8.7 ± 5.7)	461 (9.6 ± 6.8)	805 (6.8 ± 5.7)[a]	523 (8.6 ± 5.1)	959 (9.2 ± 7.3)	608 (9.3 ± 5.0)[a]	0.0006
Metaphase II oocytes (*n*)	5.7 ± 4.0	6.5 ± 4.7	6.8 ± 4.6	7.5 ± 5.1[a]	5.3 ± 5.0[ab]	6.6 ± 4.6	6.5 ± 5.5	7.0 ± 4.1[b]	0.004
Ratio of metaphase II oocytes/total oocytes	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.05
Cytoplasm granulation (%)	23.7 ± 37.5	25.1 ± 40.1	31.3 ± 39.2	26.1 ± 40.0	26.8 ± 39.4	26.9 ± 38.0	36.5 ± 40.6	20.0 ± 35.9	0.09
Central granulation of cytoplasm (%)	7.4 ± 24.0	14.5 ± 33.5	13.6 ± 29.0	12.0 ± 29.8	13.0 ± 30.5	9.2 ± 24.3	9.6 ± 25.0	7.3 ± 22.1	0.7
Vacuole in cytoplasm (%)	6.2 ± 20.8	4.0 ± 16.5	2.0 ± 8.7	1.3 ± 5.6	2.0 ± 10.5	5.5 ± 15.7	5.2 ± 17.3	8.8 ± 21.4	0.05
SER clustering (%)	0.6 ± 3.4	2.6 ± 15.8	0.8 ± 7.2	2.4 ± 14.6	3.2 ± 12.2	5.1 ± 19.0	2.0 ± 11.7	2.0 ± 10.7	0.2
Inclusion body (%)	20.4 ± 34.9	9.6 ± 25.0	19.9 ± 35.5	13.8 ± 29.2	15.7 ± 30.0	24.5 ± 34.2	25.8 ± 37.3	28.2 ± 37.6	0.01
Amorph oocyte (%)	2.1 ± 7.5	4.9 ± 16.9	6.5 ± 16.1	4.1 ± 9.0	5.0 ± 17.5	2.3 ± 7.0	5.9 ± 18.5	3.5 ± 15.1	0.2
Oval shaped oocyte (%)	0.8 ± 4.9	0.1 ± 0.8	1.7 ± 7.2	0.7 ± 4.8	2.4 ± 12.8	0.7 ± 3.0	0.9 ± 5.7	1.6 ± 6.1	0.7
Dark oocyte (%)	0	0	0.1 ± 1.1	0.3 ± 2.1	0.6 ± 4.9	0	0	0	0.2
Extended PVS (%)	30.7 ± 39.5	35.8 ± 42.1	26.7 ± 35.6	23.2 ± 34.3	39.8 ± 42.5	29.6 ± 38.7	39.8 ± 41.5	27.1 ± 39.1	0.05
Granulated PVS (%)	19.7 ± 36.5	23.1 ± 37.4	21.4 ± 35.0	19.5 ± 34.1	24.0 ± 38.9	22.5 ± 34.7	29.2 ± 38.6	24.2 ± 39.1	0.4
Multiple fragmented PB (%)	47.2 ± 41.7	34.5 ± 38.9	45.6 ± 39.9	37.8 ± 39.9	37.0 ± 39.7	50.0 ± 38.7	43.5 ± 40.2	50.3 ± 39.7	0.1
Thin ZP (%)	2.6 ± 14.4	7.1 ± 21.4	5.1 ± 18.7	3.0 ± 14.2	3.4 ± 17.4	5.10 ± 17.6	0.2 ± 1.6	2.5 ± 13.8	0.1
Thick ZP (%)	1.9 ± 11.6	4.4 ± 19.5	1.8 ± 12.1	2.9 ± 15.1	3.9 ± 18.5	0	0.2 ± 2.5	3.9 ± 18.5	0.4
Double fragmented PB (%)	0.4 ± 3.3	1.6 ± 9.5	0.5 ± 4.6	0.3 ± 1.5	0.7 ± 5.2	0.5 ± 3.7	1.6 ± 10.7	1.8 ± 12.5	0.9
Huge PB (%)	0[a]	0	0[b]	0[c]	0.04 ± 0.38[d]	0.18 ± 1.42	0[e]	2.24 ± 12.78[abcde]	0.002
AOQI	1.6 ± 1.1	1.7 ± 1.3	1.8 ± 1.1	1.5 ± 1.0	1.8 ± 1.1	1.8 ± 1.0	2.0 ± 1.2	1.8 ± 1.3	0.3

Data were presented as mean ± SD or as shown. Comparisons with the identical superscripts reveal significant differences (Regarding the Huge PB variable, the adjusted p-value of post hoc tests were as follow; a: *P*-value = 0.002, b: *P*-value = 0.006, c: *P*-value = 0.028, d: *P*-value = 0.013, e: *P*-value = 0.002, the others are > 0.05). (Regarding the total number of oocytes aspirated variable, the adjusted *P*-value of post hoc tests were as follow; a: *P*-value = 0.001, the others are > 0.05). (Regarding the Metaphase II oocytes (*n*) variable the adjusted *P*-value of post hoc tests were as follow; a: *P*-value = 0.030, b: *P*-value = 0.010, the others are > 0.05)

HCG-OPU hCG injection to oocyte pick up interval; *OPU-DN* oocyte pick up to denudation interval; *DN-ICSI* denudation to ICSI interval; *SER* smooth endoplasmic reticulum; *PVS* perivitelline space; *PB* polar body; *ZP* zona pellucida; *AOQI* oocyte quality index (total number of dysmorphisms for all metaphase II oocytes in a cycle divided by the number of metaphase II oocytes in a cycle)

Table 3 Outcomes of ICSI cycles

Variable	HCG–OPU ≤ 36 h ($n=264$)				HCG–OPU > 36 h ($n=349$)				*P*-value
	OPU–DN ≤ 2 h ($n=141$)		OPU–DN > 2 h ($n=123$)		OPU–DN ≤ 2 h ($n=180$)		OPU–DN > 2 h ($n=169$)		
	DN–ICSI ≤ 2 h ($n=101$)	DN–ICSI > 2 h ($n=40$)	DN–ICSI ≤ 2 h ($n=75$)	DN–ICSI > 2 h ($n=48$)	DN–ICSI ≤ 2 h ($n=119$)	DN–ICSI > 2 h ($n=61$)	DN–ICSI ≤ 2 h ($n=104$)	DN–ICSI > 2 h ($n=65$)	
Fertilization rate (%)	70.2 ± 29.1	69.3 ± 26.1	71.9 ± 23.2	72.6 ± 24.2	73.5 ± 29.9	69.2 ± 28.4	67.9 ± 29.6	71.5 ± 25.7	0.7
Cleavage rate (%)	91.1 ± 24.0	96.3 ± 16.5	93.6 ± 19.5	94.6 ± 17.6	86.2 ± 31.5	95.9 ± 15.4	92.2 ± 22.4	90.0 ± 27.8	0.5
Biochemical pregnancy rate per transfer (%)	31.3	38.2	34.4	18.6	33.0	30.0	28.6	25.9	0.6
Clinical pregnancy rate per transfer (%)	29.6	26.5	34.4	16.3	27.7	28.0	25.6	24.1	0.6

Data were presented as mean ± SD or percentage

HCG-OPU hCG injection to oocyte pick up interval; *OPU-DN* oocyte pick up to denudation interval; *DN-ICSI* denudation to ICSI interval

HCG–OPU interval is essential for the in vivo maturation of oocytes [15]. We schedule OPU in our unit, in a range of 35–39 h after hCG administration. After adjustment for confounders, we noticed that the prolongation of hCG-OPU interval could lead to the increased incidence of cytoplasmic granulation and inclusion body in the oocytes. Moreover, AOQI was associated with hCG–OPU interval. It has been suggested by some previous studies that oocytes with slight dysmorphisms may not alter the reproductive outcomes, while the increased number of dysmorphisms in oocyte may impair the developmental competence of embryo [14, 16]. However, in this study, no significant differences were observed in terms of fertilization, cleavage, and pregnancy outcomes in different hCG–OPU intervals. Hence, in vivo time interval between hCG and OPU might influence the quality of oocytes, but it did not affect the ICSI outcomes. However, there is a lack of evidence about the molecular and chromosomal status of embryos derived from oocytes with a high number of dysmorphisms. Previously published literature suggested 35–38 h [17] and 36–38 h [18] for the hCG–OPU interval, as the ICSI outcomes were unaffected in theses ranges of time. Nevertheless, Garor and colleagues reported that hCG–OPU interval in a range of 36–38 h could be associated with higher fertilization and pregnancy outcomes when compared to 34–36 h [7]. Pereira and colleagues reported that the hCG-OPU interval might impact successful fertilization in ICSI cycles [11]. The small sample size and different design of the latter study may explain its unusual finding. However, according to our results, the schedule of OPU can be extended from 35 to 39 h after hCG administration without any significant changes in fertilization, cleavage, and pregnancy outcomes.

Retrieved oocytes are surrounded by cumulus cells which communicate with oocytes and influence their developmental processes [3, 19, 20]. Hence, the in vitro culture of oocytes before denudation of surrounding cumulus cells may influence the development and fertilization potential of oocytes. In the current study, denudation of oocytes was performed from 0.5 to 6 h after OPU, based on the daily workload of our unit. According to our results, although the OPU-denudation interval was associated with the presence of cytoplasmic granulation and extended PVS in oocytes, there was no significant association between this interval and fertilization, cleavage, and pregnancy rates. Moreover, the association of OPU–denudation interval with granulated PVS of oocytes was clinically considerable, but it was not statistically significant. Taken together, the available evidence did not support the adverse effect of PVS dysmorphisms on fertilization and reproductive outcomes of ICSI cycle [14]. So, the timing for the in vitro culture of the aspirated oocytes could alter the oocyte quality, but it did not change subsequent reproductive outcomes. Although there is no previous report studying the association of OPU–denudation interval with oocyte dysmorphisms, our finding was in accordance with the most of previous studies indicating that OPU–denudation interval did not affect fertilization and pregnancy outcomes of ICSI [6, 7, 9, 12]. Isiklar and colleagues reported that OPU–denudation interval might affect the fertilization and cleavage rates. In the current work, this interval did not impact pregnancy outcomes of ICSI cycles; however,

Table 4 Multiple regression analysis of potential factors associated with main outcome measures

Logistic regression	Variable	OR	*P*-value	95% C.I. for OR	
				Lower	Upper
Biochemical pregnancy per ET	Female age	0.938	0.014	0.891	0.987
	No. total aspirated oocytes	0.950	0.065	0.900	1.003
Clinical pregnancy per ET	Female age	0.943	0.028	0.895	0.994
	No. total aspirated oocytes	0.953	0.093	0.901	1.008
Linear regression	Variable	*B*	SE	Standardized beta	*P*-value
AOQI	No. total aspirated oocytes	–0.035	0.012	–0.178	0.003
	HCG–OPU > 2 h	0.369	0.138	0.156	0.008
Fertilization rate	Total dose of gonadotrophin	0.002	0.001	0.101	0.089
	Male infertility	5.541	3.257	0.101	0.090
Cleavage rate	Total dose of gonadotrophin	–0.002	0.001	–0.115	0.059
	BMI	–0.685	0.389	–0.107	0.079
Poisson regression	Variable	IRR	*P*-value	95% C.I. for IRR	
				Lower	Upper
Cytoplasm granulation	HCG–OPU > 2 h	0.785	0.021	0.639	0.964
	OPU–DN > 2 h	1.248	0.006	1.088	1.669
	DN–ICSI > 2 h	1.305	0.013	1.058	1.608
	DOR	0.569	0.010	0.370	0.874
Vacuole in cytoplasm	Male infertility	0.547	0.010	0.346	0.865
	Total dose of gonadotrophin	1.000	0.048	0.999	1.000
Inclusion body	BMI	1.045	0.007	1.012	1.017
	HCG–OPU > 2 h	1.264	0.041	1.010	1.582
Granulated PVS	OPU–DN > 2 h	1.212	0.065	0.988	1.486
	AMH	0.956	0.002	0.929	0.983
Extended PVS	OPU–DN > 2 h	1.218	0.033	1.016	1.460
	Male infertility	0.799	0.014	0.668	0.955
	DOR	0.655	0.008	0.480	0.894
	RIF	1.450	0.041	1.016	2.071
Multiple fragmented PB	DN–ICSI > 2 h	1.140	0.092	0.979	1.328
	DOR	0.524	< 0.001	0.379	0.724
	BMI	1.032	0.003	1.011	1.053
	Total dose of gonadotrophin	0.999	0.036	0.999	0.999
Thin ZP	AMH	0.837	0.043	0.704	0.995
Huge PB	Primary or secondary infertility	0.024	0.005	0.002	0.323
	Male infertility	14.950	0.013	1.767	126.560
	Female age	0.818	0.038	0.676	0.989

HCG-OPU hCG injection to oocyte pick up interval; *OPU-DN* oocyte pick up to denudation interval; *DN-ICSI* denudation to ICSI interval; *ICSI* intracytoplasmic sperm injection; *hCG* human chorionic gonadotrophin; *OPU* oocyte pick up; *DOR* decreased ovarian reserve; *AMH* anti-mullerian hormone; *BMI* body mass index; *RIF* recurrent implantation failure; *PVS* perivitelline space; *PB* polar body; *ZP* zona pellucida; *AOQI* oocyte quality index (total number of dysmorphisms for all metaphase II oocytes in a cycle divided by the number of metaphase II oocytes in a cycle). No variable had a significant effect on the amorph, oval, and dark oocytes, central granulation of cytoplasm, smooth endoplasmic reticulum clustering, thin ZP, and double fragmented PB

it should be considered that they did not account for the important confounding variables in their outcomes [8]. Besides, our findings disagree with a previous report in which OPU–denudation interval could affect fertilization and pregnancy outcomes after ICSI. However, their report should be dealt with their limitation regarding the low number of studied ICSI cycles [10].

Another important interval in the ICSI cycle is the time between denudation and ICSI, in which the denuded oocytes are cultured in vitro. We hypothesized that the

prolongation of this interval might affect the developmental and fertilization potential of the oocytes. Our results demonstrated that the denudation–ICSI interval was associated with the presence of oocyte granulation in the oocytes, but it did not affect the fertilization, cleavage, and pregnancy rates. Also, it was clinically considerable that the denudation–ICSI interval was associated with the presence of multiple fragmented PB in oocytes, but this association was not statistically significant. No previous study was reported the association between the denudation–ICSI interval and oocyte dysmorphisms. Our finding regarding reproductive outcomes in different denudation-ICSI intervals is in accordance with the majority of previous reports on this topic [6, 7, 12, 21]. Patrat and colleges studied 110 ICSI cycles in a retrospective design and concluded that denudation–ICSI interval might impact the fertilization rate in ICSI cycles, but this interval did not affect the pregnancy rate [10].

The strengths of the current study are the prospective design of the work, wide ranges of time intervals, and adjustment to the confounding variables. Despite the current concern about the prolongation or shortening of the time intervals between various steps of ICSI cycle due to the workload of units, the results of the present study demonstrated that performing OPU 35 to 39 h after hCG administration, and prolongation of OPU-denudation and denudation–ICSI time intervals beyond 6 and 4.8 h, respectively, did not affect the fertilization, cleavage and pregnancy rates. Therefore, ART units for carrying out the ICSI cycles possess a wide window of time between the various steps of ICSI procedure without adverse consequences in terms of fertilization, cleavage, and pregnancy outcomes.

Conclusion

In the present study, we demonstrated that the presence of cytoplasmic granulation in oocytes retrieved after ovarian stimulation in ICSI cycles was associated with the hCG–OPU, OPU–denudation, and denudation–ICSI intervals. Besides, there were significant associations between the hCG–OPU interval and inclusion body in oocytes, and between the OPU–denudation interval and extended PVS of oocytes. The total number of dysmorphic premature oocytes were affected by the hCG–OPU interval in ICSI cycles. Hence, the oocyte dysmorphisms mainly occurred with prolongation of the in vivo maturation timing. However, the different time intervals between various steps of the ICSI procedure did not impact the fertilization, cleavage, and pregnancy outcomes. In sum, our findings suggest that the issue of timing in the ICSI cycle alters the oocyte quality, but it does not have a crucial impact on reproductive outcomes.

Acknowledgements This study is financially supported by Men's health and Reproductive Health Research Center (MHRHRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran (grant number: 12102).

Author contributions EA: protocol development, data collection, data analysis, and manuscript writing; MN: data collection, data analysis, and manuscript writing; HN: project development and data collection; SS: project development and data collection; MK: data collection and data analysis; NB: data collection and data analysis; ZSM: project development, data analysis, and manuscript editing.

Compliance with ethical standards

Conflict of interest The authors of the current paper declare no conflict of interest regarding all periods of working—neither financial support nor personal relationship. Elham Azizi declares that she has no conflict of interest. Mohammad Naji declares that he has no conflict of interest. Hamid Nazarian declares that he has no conflict of interest. Saghar Salehpour declares that she has no conflict of interest. Maryam Karimi declares that she has no conflict of interest. Nasrin Borumandnia declares that she has no conflict of interest. Zahra Shams Mofarahe declares that she has no conflict of interest.

Informed consent statement Informed consent was obtained from all participants.

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