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Obstetrics and Gynecology

# The time interval between the hCG trigger and the oocyte pickup on IVF outcomes in patients with decreased ovarian reserve and poor prognosis

Gönül Özer<sup>1,2</sup><sup>®</sup>, İpek Nur Balın Düzgüner<sup>1</sup><sup>®</sup>, Sevinç Özmen<sup>1</sup><sup>®</sup>, Semra Kahraman<sup>1</sup><sup>®</sup>

<sup>1</sup>*IVF* and Reproductive Genetics Centre, Memorial Şişli Hospital, İstanbul, Türkiye; <sup>2</sup>Department of Obstetrics and Gynecology, Faculty of Medicine, Üsküdar University, İstanbul, Türkiye

# ABSTRACT

**Objectives:** This study aimed to evaluate the effect of varying intervals between human chorionic gonadotropin (hCG) administration and oocyte pickup (OPU) at 34, 35, and 36 hours on in vitro fertilization (IVF) outcomes and embryo development in patients with diminished ovarian reserve (DOR).

**Methods:** This retrospective cohort study utilised the Istanbul Memorial Şişli Hospital, assisted reproductive technology (ART) and Reproductive Genetics Center databases from January 2017 to March 2024. The study included women undergoing ART cycles with DOR, as defined by the Bologna criteria, and follicle-stimulating hormone (FSH) levels exceeding 12 IU/L on day 2 of menstruation. Nine hundred and forty-nine ovarian stimulation cycles were analysed using the gonadotropin-releasing hormone antagonist protocol and triggered by recombinant hCG (r-hCG). The cycles were divided into three groups based on the time interval between r-hCG injection and OPU at 34, 35, and 36 hours. Demographic characteristics, ovarian stimulation parameters, embryological outcomes, and pregnancy results were compared across these groups.

**Results:** The 36-hour OPU group demonstrated the highest fertilization, best embryological outcomes, including the highest blastocyst formation rate (14.95%) compared to the 34-hour (14.23%) and 35-hour (12.43%) groups (P=0.025). The 36-hour group also had the highest proportion of day 5–6 embryo transfers (33.8%, P=0.001). However, there were no significant differences in pregnancy outcomes.

**Conclusions:** In DOR patients, extending the OPU interval to 36 hours with hCG triggering showed higher fertilization rates and better embryo development than 34 and 35 hours. However, it did not affect pregnancy outcomes.

**Keywords:** Oocyte pickup (OPU), diminished ovarian reserve (DOR), in vitro fertilization (IVF), pregnancy outcomes

he management of in vitro fertilization (IVF) procedures in patients with diminished ovarian reserve (DOR) presents significant challenges for clinicians. These patients often exhibit limited retrievable oocytes, poor oocyte quality, and

suboptimal response to ovarian stimulation. These factors contribute to reduced fertilisation rates, lower pregnancy success rates, and impaired embryo development [1]. Among the critical determinants of successful IVF outcomes in this population are oocyte

Corresponding author: Gönül Özer, MD., Assist. Prof., Phone: +90 212 314 66 66, E-mail: drgonulozer@gmail.com

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maturation and quality, which depend on precise timing during assisted reproductive technology (ART) cycles [2].

The relationship between physiological ovulation timing and luteinizing hormone (LH) elevation highlights the importance of precise timing in ART cycles. Ovulation, the release of an oocyte from the follicle, usually occurs 34-36 h after the LH surge onset or 10-12 h following the LH peak. This timing is critical to ensure that the oocyte reaches the mature metaphase II (MII) stage, which is essential for successful fertilisation in both natural and assisted reproductive cycles. However, individual variability in LH secretion patterns, follicular responsiveness, and hormonal feedback mechanisms can significantly influence ovulation timing. Physiological studies have demonstrated that ovulation can occur within 24 and 56 h after the LH surge onset, with an average duration of 32 h [3].

In controlled ovarian stimulation (COS) protocols, exogenous human chorionic gonadotropin (hCG) is given to simulate the LH surge and induce a similar physiological cascade. The timing of oocyte pickup (OPU) relative to HCG administration is critical for retrieval of mature oocytes. If OPU is performed too early after the hCG trigger, oocytes may stay in the germinal vesicle (GV) or metaphase I (MI) stages, resulting in reduced fertilization potential [4]. Conversely, if OPU is delayed beyond the optimal window, there is an increased risk of premature ovulation, which can lead to the loss of oocytes into the peritoneal cavity or post-maturation changes that compromise oocyte quality and subsequent embryo development [5].

While studies have explored the impact of trigger to OPU intervals in general IVF populations, limited data are available on this parameter in patients with DOR and poor prognosis. These patients, often characterised by low oocyte yield and suboptimal quality, may be particularly susceptible to slight timing variations. Understanding the complex relationship between the trigger to OPU interval and oocyte development is crucial for creating customised protocols to enhance outcomes for this challenging subgroup.

## **METHODS**

**Study Population** 

This retrospective cohort study utilised the Şişli Memorial Hospital, ART, and Reproductive Genetics Center databases from January 2017 to March 2024. The study included women undergoing ART cycles with diminished ovarian reserve, according to the Bologna criteria, and follicle-stimulating hormone (FSH) levels exceeding 12 IU/L on day 2 of menstruation. Patients with congenital or acquired uterine abnormalities, a history of thin endometrium, or FSH levels above 20 IU/L on day 2 of the menstrual cycle were excluded. In total, 949 ovarian stimulation cycles employing the gonadotropin-releasing hormone (GnRH) antagonist protocol and triggered by recombinant human chorionic gonadotropin (r-hCG) were analysed. In patients whose LH levels begin to rise on the day of triggering or whose estradiol (E2) values drop the day after triggering, the OPU time is scheduled for 34 or 35 hours rather than 36 hours. Cycles were categorized into three groups based on the time interval between r-hCG injection and OPU: group one (34 h), group two (35 h), and group three (36 h). The groups were compared in terms of demographic characteristics, ovarian stimulation cycle parameters, embryological data, and pregnancy outcomes. The ethical approval was obtained from the Institutional Review Board of Istanbul Memorial Şişli Hospital, Istanbul, Türkiye (approval number: 29.04.2024/002).

#### **Ovarian Stimulation Protocol**

Ovarian stimulation was started on the second day of menstruation using the antagonist protocol. The initial dose of gonadotropins was adjusted based on body mass index (BMI), number and size of antral follicles, and relevant ultrasound and hormonal findings. Ovarian stimulation was conducted using one of the following medications: human menopausal gonadotropin (hMG) (Menogon®, Ferring, Switzerland), a combination of recombinant follicle-stimulating hormone (rFSH) (Gonal-F®, Merck Serono, Switzerland) with recombinant luteinizing hormone (rLH) (Pergoveris®, Merck Serono, Switzerland), or rFSH alone. The timing of ovulation triggering was determined based on follicle size and serum hormone levels. Ovulation was induced using r-hCG (Ovitrelle®, Merck Serono, Switzerland).

# **Oocyte Pickup and Intracytoplasmic Sperm Injection (ICSI)**

Oocyte Pickup was performed 34-36 h after the trigger under sterile conditions using transvaginal ultrasonography guidance. 16-17G diameter aspiration needle was employed with a negative pressure of approximately 120-130 mmHg. Retrieved oocytes were sent to the embryology laboratory in Earle's Balanced Salt Solution (EBSS) flushing medium (Multicell, Wisent INC, Québec, Canada). Oocytes were observed under  $100 \times$  magnification using HEPES-buffered medium and transferred to culture medium (LifeGlobal, Cooper Surgical, Brussels, Belgium). Oocytes were typically denuded within three h of aspiration and assessed for maturity. Mature oocytes were subjected to Intracytoplasmic Sperm Injection (ICSI) within 1 h of denudation.

#### **Embryo Grading**

Day 3 embryos were graded using Gardner's classification based on blastomere number, symmetry, and fragmentation. Grade 1 embryos were characterised by blastomeres of equal size, fragmentation of less than 10%, and a homogeneous cytoplasm. Grade 2 embryos had slight asymmetry and 10%-20% fragmentation. Grade 3 embryos showed more asymmetry and more than 20% fragmentation. Grade 4 embryos had high fragmentation and marked asymmetry. Blastocysts also using Gardner's classification are categorised as follows: Top quality (TQ): Hatched AA, 6AA, 5AA, 4AA; Good quality (GQ): Hatched AB/BA/BB, 5AB/BA/BB, 4AB/BA/BB, 3AA: Medium quality (MQ): 3AB/BA, 2AA; Poor quality (PQ): others.

#### **Luteal Phase Support**

For luteal phase support, vaginal progesterone gel (Crinone 8%, 90 mg; Merck Serono, Switzerland) was administered twice daily from January 2017 to November 2020. After November 2020, the luteal support protocol was modified due to the Crinone gel's commercial unavailability. Patients were then prescribed daily subcutaneous progesterone injections (Progestan Dex 25 mg/mL, Koçak, Türkiye) along with vaginal progesterone tablets (Progestan 100 mg, Koçak, Türkiye) administered three times daily.

#### **Statistical Analysis**

The study provided descriptive statistics as means

and standard deviations for quantitative variables and as numbers and percentages for qualitative variables. The normal distribution of quantitative variables was assessed graphically using the Kolmogorov-Smirnov test. The Anova test was used to compare differences between OPU groups. The relationship between qualitative variables and OPU groups was analysed using the Pearson's chi-square test. Statistical analyses and calculations were performed using IBM SPSS Statistics 27.0 (IBM Corp., Released 2020; IBM SPSS Statistics for Windows, Version 27.0; Armonk, NY: IBM Corp.) and MS Excel 2016. Statistical hypotheses were evaluated with a Type I error level of  $\alpha$ =0.05.

## RESULTS

The IVF cycle parameters and demographic data of the OPU groups are shown in Table 1. The anti-Mullerian hormone (AMH) levels and antral follicle counts were lowest in the 35-hour group and highest in the 36-hour group, while LH and FSH levels were lowest in the 36-hour group and highest in the 34-hour group. The daily gonadotropin dose was lowest in the 36-hour group and highest in the 35-hour group, whereas the total gonadotropin dose and COS length were lowest in the 36-hour group and highest in the 34-hour group. The estradiol level on trigger day was lowest in the 34-hour group and highest in the 36-hour group. The PN2 values were lowest in the 34-hour group and highest in the 36-hour group (P<0.05). A significant negative relationship was found between OPU timing and embryo transfer (ET) cancellation, OPU cancellation, and ET cancellation groups (P<0.05). The study compared embryological outcomes across 34h, 35h, and 36h OPU groups. Day 3 grade 1-2 embryo rates were similar (P=0.185), but blastocyst formation rate was highest in the 36-hour group (14.95%), followed by 34-hour (14.23%) and 35-hour (12.43%) (P=0.025). The proportion of topquality and good-quality blastocysts on day 5 was significantly higher in the 34-hour (12.82%) and 36-hour (12.81%) groups than in the 35-hour group (10.55%)(P=0.029). A significantly increased rate of day 5-6 embryo transfers was observed in the 36-hour group (33.8%) compared to the 34-hour (14.8%) and 35-hour (18.3%) groups (P=0.001). However, single vs. double

Table 1. Comparison of patient demo	Group 1	Group 2	Group 3	P value
	34.h OPU	35.h OPU	36.h OPU	
	(n= 83)	(n= 231)	(n= 635)	
Female age (years)	39.50±5.35	38.16±4.97	39.12±4.06	0.581
Female BMI (kg/m <sup>2</sup> )	$21.03 \pm 1.37$	$24.09 \pm 3.81$	23.63±3.73	0.058
Duration of infertility (years)	$3.75 \pm 2.50$	$4.66\pm3.70$	4.32±3.97	0.218
Number of previous cycles	5.25±3.30	2.95±3.21	$5.04 \pm 3.74$	0.182
AMH (ng/mL)	0.33±0.13	$0.36 \pm 0.24^{b}$	$0.38{\pm}0.31$	<0.001*
LH level on the trigger day (IU/L)	$13.46{\pm}10.00^{a}$	8.54±6.43	$5.85 \pm 9.42^{\circ}$	<0.001*
<b>Basal antral follicle count</b>	$1.50 \pm 0.58$	$1.63 \pm 0.76^{b}$	$2.24{\pm}1.41$	0.012*
FSH level on the second day of menstruation (IU/L)	19.50±11.93 <sup>a</sup>	15.53±2.04	15.35±2.68	<0.001*
Daily gonadotropin dosage used (IU)	261.67±64.68	274.33±97.65	259.42±83.54 <sup>c</sup>	<0.001*
Totally gonadotropin dosage used (IU)	1856.25±663.44	2211.84±1426.20	2147.60±1270.83°	<0.001*
Duration of controlled ovarian stimulation (days)	7.00±1.83	7.68±2.77	7.93±2.59°	0.003*
Estradiol level on trigger day (pg/mL)	433.75±267.67	572.05±363.09	637.45±484.03°	<0.001*
Number of aspirated oocytes, (COC)	$1.00{\pm}0.00$	$1.89{\pm}1.45$	$2.27{\pm}1.40$	0.080
Number of MII	$1.00{\pm}0.00$	$1.58 \pm 1.31$	2.02±1.25	0.079
Number of PN2	$0.50{\pm}0.58$	1.16±1.12 <sup>b</sup>	$1.63 \pm 1.05$	0.003*
Oocyte maturation rate (%)	83.2±20.53	88.42±21.61	92.37±17.23	0.047*
Fertilization rate (%)	$50.00 \pm 57.74$	78.07±41.59	$82.92 \pm 29.87$	0.038*
OPU cancellation rate (premature ovulation)	13/83 (15.7)	8/231 (3.5)	14/635 (2.2)	<0.001*
ET cancellation rate	44/70 (62.9)	103/223 (46.2)	309/621 (49.8)	0.049*
Causes of ET cancellation, n (%)				
All freeze (other reasons)	9/44 (20.5)	11/103 (10.7)	44/309 (14.2)	
PGT/All freeze	9/44 (20.5)	33/103 (32.0)	146/309(47.2)	
No oocyte	8/44 (18.2)	10/103 (9.7)	19/ 309 (6.1)	
No mature oocyte	4/44 (9.1)	6/103 (5.8)	10/ 309 (3.2)	
Fertilization failure	3/44 (6.8)	20/103 (19.4)	38/ 309 (12)	
Embryo developmental arrest	6/44 (13.6)	15/103 (14.6)	34 /309 (11.0)	
Degenerated oocyte	0 (0.0)	0 (0.0)	3/309 (1.0)	
Abnormal fertilization	1/44 (2.3)	2/103 (1.9)	8 /309(2.6)	
PN arrest	4/44 (9.1)	6/103 (5.8)	7/309 (2.3)	

# Table 1. Comparison of patient demographics and IVF cycle characteristics

Data are shown as mean±standard deviation or n (%). IVF=In vitro fertilization, OPU=Oocyte pickup, BMI=Body mass index, AMH=Anti-mullerian hormone, LH=Luteinizing hormone, FSH=Follicle stimulating hormone, COC=Cumulus-oocyte complex, MII=Metaphase II, PN=Pronuclear, ET=Embryo transfer, PGT=Preimplantation genetic testing <sup>a</sup>There was a significant difference from the other groups.

<sup>b</sup>There was a significant difference from Group 3.

<sup>c</sup>There was a significant difference from the other groups.

Comparison of embryologic developmental characteristics of ICSI cycles					
	Group 1 34.h OPU (n=73)	Group 2 35.h OPU (n=223)	Group 3 36.h OPU (n=621)	P value	
D3 grade 1-2 embryo ratio (%)	33.65	37.35	32.87	0.185	
Blastocyst ratio (%)	14.23	12.43	14.95	0.025*	
D5 TQ-GQ ratio (%)	12.82	10.55	12.81	0.029*	
Comparison of the characteristics of embryo transfer cycles					
	Group 1 34.h OPU (n=27)	Group 2 35.h OPU (n=126)	Group 3 36.h OPU (n=352)	P value	
Day of embryo transfer, n (%)				0.001*	
D3-4	23 (85.2)	103 (81.7)	233 (66.2)		
D5-6	4 (14.8)	23 (18.3)	119 (33.8)		
Number of embryos transferred, n (%)				0.576	
Single	22 (81.5)	92 (73.0)	254 (72.2)		
Double	5 (18.5)	34 (27.0)	98 (27.8)		

## Table 2. Comparison of embryological development and transfer characteristics in ICSI cycles

embryo transfer rates did not differ significantly (P=0.576) (Table 2). Pregnancy outcomes showed no significant differences between the groups (Table 3).

## DISCUSSION

Our study investigated the impact of OPU timing conducted at 34, 35, and 36 h after hCG trigger on ovarian response parameters, embryologic outcomes, and cycle cancellation rates in patients with poor prognosis. The significant differences observed among the groups suggest that optimising OPU timing can improve outcomes, particularly in patients with a poor prognosis. While female age and body mass index (BMI) did not show significant differences between the groups, significant differences were observed in AMH levels, and the 36-hour OPU group was found to have higher AMH values. This indicates that patients in this group had a relatively better ovarian reserve than those in the earlier OPU groups. Similarly, the basal antral follicle count was significantly higher in the 36-hour group, further supporting enhanced ovarian capacity in this cohort [6, 7]. Additionally, FSH levels were significantly found higher in the 34-

hour OPU group, which is consistent with a DOR and may explain the lower oocyte yield in this subgroup. Oocyte maturation and fertilisation rates were significantly higher in the 36-hour OPU group, suggesting that prolonging the trigger-OPU interval may increase oocyte yield and maturation and thus affect fertilisation. Several studies have supported our findings [8-12]. For instance, Wang et al. [8] performed a meta-analysis showing that extending the interval between hCG priming and oocyte retrieval can lead to an increase in the percentage of MII oocytes. Similarly, Garor et al. [9] reported higher fertilisation, embryo transfer, and pregnancy rates when oocyte pick-up was performed after 36 hours post-trigger, particularly in GnRH agonist cycles Furthermore, Shen et al. [10] identified an optimal trigger-to-pickup interval of 36.4-37.8 hours in progestin-primed ovarian stimulation cycles, which was correlated with higher rates of mature oocytes, improved implantation rates, and increased live birth rates. Weis et al.'s [13] study found that fewer MII oocytes were retrieved when the period between oocyte triggering and OPU was between 33.45 and 34.45 hours. They observed an increase in the MII oocyte rate with a 35-hour delay, which then stabilized up to 38 hours. However,

Table 5. Comparison of pregnancy outcomes per emory of ransfer				
	Group 1 34.h OPU (n=27)	Group 2 35.h OPU (n=126)	Group 3 36.h OPU (n=352)	P value
Biochemical pregnancy rate, n (%)	6/27 (22.2)	33/126 (26.2)	115/352 (32.7)	0.252
Clinical pregnancy rate, n (%)	6/27 (22.2)	31/126 (24.6)	92/352(26.1)	0.867
Total pregnancy loss rate, n (%)	2/6 (33.3)	15/33 (45.5)	50/115(43.5)	0.859
Live birth rate, n (%)	4/27 (14.8)	15/126 (11.9)	61/352(17.3)	0.338

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other studies report contrasting results [14-16]. For instance, Nargund et al.'s [14] prospective study found no significant differences in oocyte maturation between OPU procedures performed 33 to 41 hours posttrigger. Bosdou et al. [15] showed that in normo-ovulatory women, extending the interval between HCG administration and oocyte collection from 36 to 38 hours did not significantly affect the oocyte collection rate, fertilization rate, or live birth rate. In this study, in contrast to our study, the study group consisted of normovulatory patients [159. Ranit et al. [16] analysed 438 cycles and showed that GnRHa trigger and OPU interval between 32.03 and 39.92 h did not significantly affect oocyte yield and maturation rat. These findings suggest that while optimising timing may benefit certain patient groups, its impact could vary based on specific stimulation protocols and individual patient characteristics. In our study, the 36-hour OPU group had the highest fertilization rate (82.92%), followed by the 35-hour (78.07%) and 34-hour (50.00%) groups. In contrast to our study, a metaanalysis by Wang et al. [8] found that a longer interval between hCG triggering and OPU (>36 hours) increased MII oocyte yield but did not significantly improve fertilisation, implantation, or pregnancy rate. Gan et al. [3] also found no significant differences in oocyte maturation, fertilisation, or high-quality embryo rates between short ( $\leq$ 36h) and long (>36h) hCG-OPU intervals. There are also studies with results compatible with our study. Choi et al. [17] reported higher fertilisation rates at 36th hour, which is compatible with our findings. Garor et al. [9]also associated delayed OPU with more embryos and higher fertilisation than early OPU. An important observation in this study was the significantly higher OPU cancellation rate due to early ovulation in the 34 hour OPU

group (15.7%) compared to the 35-hour (3.5%) and 36-hour (2.2%) groups (P<0.001). In the 34-hour OPU group, we aimed to reduce the risk of premature ovulation by scheduling the OPU procedure earlier due to the high LH level on the trigger day. However, early OPU did not reduce the risk of premature ovulation in this group and resulted in fewer mature oocytes and lower fertilisation rates. In line with our study findings, Choi *et al.* [17] reported that early oocyte retrieval during an early LH surge did not effectively reduce cycle cancellation rates and may lead to lower fertilisation rates.

Furthermore, embryo development parameters were superior in the 36-hour OPU group, reinforcing the potential advantage of extended OPU timing. Some studies in the literature support our results [11, 18]. Skvirsky et al. [11] showed that extending the time interval between hCG administration and OPU may improve oocyte maturation and embryo quality in women over 36 years of age. The blastocyst formation rate was significantly different among the groups (P=0.025), with the highest rate observed in the 36hour OPU group. This finding suggests that delaying OPU to 36 h may benefit blastocyst development, possibly due to improved oocyte maturity and cytoplasmic competence. Similarly, the D5 TQ-GQ embryo ratio was significantly different between the groups (P=0.029), with the highest values in the 36-hour group. These findings indicate that a longer interval between the trigger and OPU might improve embryo quality at later stages. In our study, the 34-hour OPU group had higher rates of early ovulation, PN arrest, increased immature oocyte rates, and embryo transfer cancellation due to failure to obtain oocytes. There are many studies supporting our findings [9, 12, 17]. According to our findings, there was no statistically significant differences between the groups in terms of biochemical pregnancy rate (P=0.252), clinical pregnancy rate (P=0.867), total pregnancy loss rate (P=0.859), or live birth rate (P=0.338). While there was a trend toward higher biochemical and clinical pregnancy rates in the 36-hour OPU group, the lack of statistical significance indicated that OPU timing variations may not significantly impact pregnancy outcomes. In line with our findings, Wang *et al.* [8] reported that the timing of OPU has not significant impact on pregnancy rates. However, other studies have demonstrated improved pregnancy outcomes with later OPU timings [9, 12, 18].

# CONCLUSION

Our study highlights the significant impact of OPU timing on ovarian response, embryologic outcomes, and cycle cancellation rates in patients with poor prognosis. The findings suggest prolonging the hCG trigger to OPU interval to 36 hours may enhance oocyte yield, maturation, and embryo development. However, despite these advantages, pregnancy and live birth rates were not statistically significant different among the groups, indicating that while optimising OPU timing may improve laboratory outcomes, its effect on clinical pregnancy remains unclear.

These results align with some studies in the literature, while contradicting others, emphasising the complexity of OPU timing and its dependence on stimulation protocols and individual patient characteristics. Given the variability in outcomes, further prospective, randomised studies are necessary to determine the optimal OPU timing, particularly in patients with DOR and a poor prognosis, to maximise both embryologic and clinical success.

#### Ethical Statement

The study was approved by Memorial Şişli Hospital Ethics Committee (Decision date and no: 29.04.2024/002).

#### Authors' Contribution

Study Conception: SK, GÖ, İNBD, SÖ; Study Design: GÖ, İNBD, SÖ; Supervision: SK, GÖ, İNBD, SÖ; Funding: GÖ, İNBD, SÖ; Materials: GÖ, İNBD, SÖ; Data Collection and/or Processing: GÖ, İNBD, SÖ; Statistical Analysis and/or Data Interpretation: GÖ, İNBD, SÖ; Literature Review: GÖ, İNBD, SÖ; Manuscript Writer: GÖ, İNBD, SÖ; Critical Review: GÖ, İNBD, SÖ.

#### Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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## Editor's note

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