Testing the role of unstimulated in vitro maturation for potential development of immature oocytes in women with oocyte maturation abnormalities

Şenol Kalyoncu[1], Alper Başbuğ[2], Ebru Hatırnaz[3], Aşkı Ellibeş Kaya[4], Nur Dokuzeylül Güngör[5], Sebati Sinan Ürkmez[6], Yeşim Civil Ürkmez[7], Şafak Hatırnaz[3]

[1]Department of Obstetrics and Gynecology, Hasan Kalyoncu University Faculty of Medicine, Gaziantep, Turkey
[2]Department of Obstetrics and Gynecology, Düzce University Faculty of Medicine, Düzce, Turkey
[3]Department of Obstetrics and Gynecology, Mediliv Medical Center, Samsun, Turkey
[4]Department of Obstetrics and Gynecology, Private Office, Samsun, Turkey
[5]Department of Obstetrics and Gynecology, Bahçeşehir University Faculty of Medicine, İstanbul, Turkey
[6]Department of Biochemistry, Ondokuz Mayıs University Faculty of Medicine, Samsun, Turkey
[7]Clinic of Biochemistry, University of Health Sciences Turkey, Samsun Training and Research Hospital, Samsun, Turkey

Abstract

Objective: The aim of this study was to investigate the developmental potential of immature oocytes and evaluate whether unstimulated in vitro maturation (IVM) could serve as a treatment option for women with oocyte maturation abnormalities (OMAs).

Material and Methods: This cohort study was conducted between September 2019 and December 2022, and included women who underwent unstimulated, non-human chorionic gonadotropin (hCG) priming IVM. Oocytes were incubated with IVM medium for 26-48 hours and evaluated to compare their maturation profiles with the immature oocytes retrieved from the same patients in their previous in vitro fertilization cycles.

Results: Among the twelve women in the study, eleven (91.6%) underwent whole exome sequencing analysis. Of these, 18 variants were identified in 10 individuals, excluding case 1, who had no previous mutation analysis. Of the mutations identified, 9 (50%) were located in *FSHR*, 5 (27.8%) in *TUBB8*, 1 (5.6%) in *ZP1*, 1 (5.6%) in *SLFN14*, 1 (5.6%) in *AR*, and 1 (5.6%) in *STEAP3*. Apart from one woman with resistant ovary syndrome (ROS), none treated with unstimulated IVM had oocyte maturation. Remarkably, the only patient to achieve oocyte maturation in an unstimulated IVM cycle was case 11, who had ROS and a single *FSHR* variant.

Conclusion: Unstimulated, non-hCG primed IVM does not appear to be effective in the treatment of OMAs, perhaps with the exception of women with ROS. However, this study led our team to develop novel treatment options based on physiological mechanisms for some subtypes and supraphysiological approach for other subtypes of OMAs. (J Turk Ger Gynecol Assoc. 2024; 25: 219-23)

Keywords: Oocyte maturation arrest, oocyte maturation abnormalities, unstimulated in vitro maturation, mutation

Received: 28 December, 2023 **Accepted:** 08 July, 2024

Introduction

Recurrent immature oocyte retrieval in at least two consecutive in vitro fertilization (IVF) cycles is defined as oocyte maturation abnormalities (OMAs) (1). OMAs were initially described by Rudak et al. (2) as an oocyte factor in four cases. Subsequently, Levran et al. (3) reported different types of OMAs in eight women with unexplained infertility. Hourvitz et al. (4) defined OMA as “bad egg syndrome” and reported the first pregnancies from women with genuine empty follicle syndrome (G-EFS). Beall et al. (5) and Hatirnaz et al. (6) further defined the subtypes of OMA by excluding G-EFS, resistant ovary syndrome

Address for Correspondence: *Aşkı Ellibeş Kaya*
e.mail: askiellibes@hotmail.com ORCID: orcid.org/0000-0002-1323-7416
DOI: 10.4274/jtgga.galenos.2024.2023-10-9

(ROS), and premature ovarian failure (POF), and included only the cases with intrinsic, that is mutation-related, factors. Galvão et al. (7) reported the first pregnancies from ROS cases through in vitro maturation (IVM) in their report.

Recent publications have provided support for the notion that OMAs extend beyond OMA. The spectrum of OMAs has been expanded to include oocyte degeneration, oocyte dysmorphism, EFS, ROS, certain forms of premature ovarian insufficiency or POF, zygotic cleavage failure, and early embryonic arrest (1,8,9).

The objective of this study was to investigate the developmental potential of oocytes obtained through unstimulated, unprimed IVM from women with OMA.

Material and Methods

This retrospective cohort study was conducted between September 2019 and December 2022, and it included women who underwent unstimulated, non-human chorionic gonadotropin (hCG) priming IVM. The study received approval from the Ethical Committee of Medicana Samsun International Hospital (approval number: 7159, date: 27.12.2021). Written informed consent was obtained from all women with OMAs for all procedures. These procedures were recorded in their respective files.

The selected patients for this study had a history of recurrent OMAs in at least two IVF cycles. Patients were selected to cover the spectrum of OMAs as far as possible. However, patients with G-EFS were not included in the evaluation during the study period, despite the fact that IVM is considered the gold standard treatment for G-EFS.

The primary objective of this study was to demonstrate the developmental potential of oocytes in unstimulated IVM procedures and to evaluate the distribution of mature and immature oocytes after the IVM process.

Unstimulated IVM refers to the IVM of oocytes retrieved from natural cycles, with or without the use of an hCG trigger, as defined by Dahan et al. (10). In this study, we used unstimulated IVM (without an hCG trigger) to assess the developmental potential of immature oocytes obtained from women with OMAs. The laboratory procedures employed for OMAs in this study followed the standard protocols used in previous IVM studies (11).

Statistical analysis

The data in the study were analyzed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp, Armonk, NY, USA). In the tables, the quantitative data are presented as mean ± standard deviation and median (minimum-maximum) values, and the categorical data as number (n) and percentage (%). Mann-Whitney U test was used to compare the independent groups, and Pearson's chi-square test and Fisher's exact test to compare the categorical variables. Data were determined at the 95% confidence level, and a $p<0.05$ was accepted as statistically significant.

Results

During the study period, a total of 12 women were enrolled for unstimulated IVM after previous unsuccessful IVF cycles. Table 1 presents the demographic, laboratory, and clinical data of the patients.

Patients with OMAs, ranging from necroptosis to *TUBB8* mutation, were enrolled in the study. Among these twelve, eleven (91.6%) underwent whole exome sequencing (WES) analysis. Of these eleven, 18 variants were identified in ten women. The exception was, case 1 who had no previous mutation analysis. Interestingly, case 6, who had a history of necroptosis, did not show any detected mutations (8.3%) in the WES analysis.

Of the mutations identified, 9 (50%) were in *FSHR*, 5 (27.8%) in *TUBB8*, 1 (5.6%) in *ZP1*, and 1 (5.6%) each in *SLFN14*, *AR* and *STEAP3*. Case 4, who exhibited variable oocyte dynamics and an unclassified form of OMAs, had two *FSHR* mutations. Similarly, case 7, experiencing metaphase I-metaphase II (MI-MII) arrest, also presented two *FSHR* mutations. In cases 5 and 8, two *FSHR* mutations and one *TUBB8* mutation were detected, respectively. Case 2, who experienced MI arrest in previous IVF attempts, showed both an *AR* and a *TUBB8* mutation.

Table 1. Demographic, laboratory, and clinical data of patients[a]

	Patients (n=12)
Female age, years	32.25±4.11
Male age, years	34.50±5.46
Time of marriage, years	6.87±4.34
Infertility duration, years	6.41±4.40
BMI, kg/m^2	38.70±7.47
Basal serum FSH, IU/L	16.86±5.68
Basal serum LH, IU/L	12.47±6.12
Basal serum estradiol, pg/mL	59.49±50.55
Basal serum progesterone, ng/mL	0.59±0.36
Basal serum TSH, mU/mL	1.43±0.43
Basal serum AMH, ng/mL	2.54±1.77
Basal serum prolactin, ng/mL	20.54±13.43
AFC	12.58±7.6

[a]Data are given as mean±standard deviation. BMI: Body mass index, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone, AMH: Anti-Müllerian hormone, AFC: Antral follicle count

Table 2. Distribution of mutations in twelve women with OMAS

Patients	Diagnosis (OMAS)	Mutation 1	Gene 1	Nucleotide 1	Mutation 2	Gene 2	Nucleotide 2	Mutation 3	Gene 3	Nucleotide 3
Case 1	MI arrest	Not analyzed	-	-	-	-	-	-	-	-
Case 2	MI arrest	TUBB8	*Exon 4*	C.400C>T	AR	*Exon 4*	C.1913A>G	-	-	-
Case 3	GV arrest/zonafree	ZP1	*Exon12*	C1775-3C>A	-	-	-	-	-	-
Case 4	GV-MII arrest	FSHR	*Exon 10*	C.919G>A	FSHR	*Exon 10*	C.2039G>A	-	-	-
Case 5	GV-MI arrest	TUBB8	*Exon 4*	C.928T>C	FSHR	*Exon 10*	P.5680N	FSHR	*Exon 10*	P.A307T
Case 6	Gv arrest/necroptosis	Normal genome	-	-	-	-	-	-	-	-
Case 7	MI-MII arrest	FSHR	*Exon 10*	C.919G>A	FSHR	*Exon 10*	C.2039G>A	-	-	-
Case 8	Mixed arrest	TUBB8	*Exon 4*	C.721C>t	FSHR	*Exon 10*	P.5680N	FSHR	*Exon 10*	P.A307T
Case 9	GV arrest/zonafree	TUBB8	*Exon 4*	C.959G>A	-	-	-	-	-	-
Case 10	POF	SLFN14	*Exon 3*	C.1513A>T	STEAP3	*Exon 4*	C.907G>A	-	-	-
Case 11	ROS	FSHR	*Exon 10*	C.1412T>G	-	-	-	-	-	-
Case 12	Mixed arrest	TUBB8	*Exon 4*	C.535G>A	-	-	-	-	-	-

GV: Germinal vesicle, MI: Mitosis I, MII: Mitosis II, POF: Premature ovarian failure, ROS: Resistant ovary syndrome, TUBB8: Tubulin beta 8B, ZP1: Zona pellucida glycoprotein 1, FSHR: Follicle-stimulating hormone receptor, SLFN14: Schlafen family member 14, AR: Androgen receptor, STEAP3: Six-transmembrane epithelial antigen of prostate 3-metalloreductase

Remarkably, the only patient to achieve oocyte maturation in an unstimulated IVM cycle was case 11, who had ROS and a single *FSHR* mutation. On the other hand, case 10, the patient with POF and very small preantral follicles resistant to previous IVF attempts did not yield any oocytes in the unstimulated IVM cycle and harbored *SLFN14* and *STEAP3* variants.

Lastly, case 3 and case 9, diagnosed with germinal vesicle (GV) arrest and a zonafree phenotype had only one *ZP1* and *TUBB8* mutation, respesctively, while case 12 exhibited only a *TUBB8* mutation in the WES analysis. Table 2 provides a summary of the distribution of these mutations.

Discussion

This study revealed that immature oocytes obtained from women with OMAs exhibit limited developmental potential. In addition, it was observed that the distribution of immature oocytes in IVF cycles showed more progress compared to unstimulated, non-hCG primed IVM. These findings suggest that ovarian stimulation, whether mild or standard, might have a positive impact on the developmental potential of oocytes in women with OMAs.

The initial report by Rudak et al. (2) in 1990 was significant as it expanded the understanding of OMAs beyond the conventional classifications of GV, MI, and MII arrest studied and classified by Beall et al. (5) and Hatirnaz et al. (1). Since then, recent studies have explored the genetic variants that contribute to a wider spectrum of OMAs (5,8,12).

Levran et al. (3) conducted a study on OMAs in ICSI cycles involving eight women with history of unexplained infertility. The study identified one case of GV arrest, four cases of MI arrest, and three cases of MII arrest, which were categorized under the term “oocyte factor”. Furthermore, the study also noted atypical findings, such as one MI arrest case with four GV and 17 MI oocytes, and one MII arrest case with 13 MII and 2 MI oocytes, which did not fit into the conventional classification of oocyte maturation profiles.

The golden era of IVM was between 2000 and 2013 until when the ASRM practice committee released an opinion paper that IVM was an experimental procedure (ASRM 2013 Practice Committee). Within that time frame, many studies were conducted on the use of IVM in other indications and many treatment modalities were used rather than unstimulated IVM (13).

The first papers related to the use of IVM in women with OMAs were published in 2010 (4,5). Hourvitz et al. (4) reported on seven women who had experienced three failed IVF attempts due to “oocyte-related factors” before undergoing scheduled IVM cycles. In these cases, all women received FSH-hCG primed IVM cycles prior to oocyte retrieval, and the Canadian IVM protocol was followed for clinical and laboratory procedures (14).

Notably, this report was the first to include EFS as part of the spectrum of OMAs.

Beall et al. (5) introduced the first classification system for OMA, which was derived from analysis of previous case reports and animal studies. This classification system categorized OMAs into four distinct subtypes: type 1, characterized by GV arrest; type 2, denoting MI arrest; type 3, representing MII arrest; and type 4, encompassing mixed arrest (5). The present study primarily focused on intrinsic factors associated with OMAs and did not include other etiological factors related to OMAs. Subsequently, Hatirnaz et al. (6) recognized the limitations of the Beall et al. (5) classification system and so developed the Hatirnaz et al. (6) and Dahan et al. (10) definition system. This novel classification excluded other factors related to OMAs in their subsequent studies and reported outcomes of FSH-hCG primed IVM cycles. However, their studies did not report any clinical pregnancies, and the oocyte maturation profiles did not significantly differ from the previous IVF cycles of the same women (6).

Galvão et al. (7) conducted a study including 28 patients who underwent 49 IVM cycles to evaluate the impact of IVM in patients with ROS and women with deficient oocyte maturation. Among them, nine patients had ROS and underwent 24 IVM cycles. From these cycles, 23 cleavage-stage embryos were obtained, and eight patients achieved pregnancy, resulting in five healthy live births. The remaining 19 patients had OMAs and underwent 25 IVM cycles. In 11 of these cycles, oocyte retrieval was not successful. In 10 cycles, mature oocytes were retrieved, but fertilization failed after ICSI. Fertilization occurred in only four of the OMAs cycles, resulting in a single good quality embryo transfer, which ultimately led to a negative beta-hCG test. No live births were reported among the OMAs cases. Based on their findings, IVM may be a valuable option for women with ROS. However, they recommended caution and further improvements in the procedure before considering IVM as a suitable option for women with OMAs.

Prior to 2016, research on genetic mutations associated with OMAs primarily relied on animal studies. However, significant advances have been made since then in understanding the mechanisms and phenotypical characteristics of human genetic mutations linked to OMAs. This progress is evident in the growing number of publications focusing on human genetic variants associated with OMAs (9,15,16).

The results of mutation analysis have provided insights into the genetic basis of various forms of OMAs. Interestingly, certain severe forms of OMAs, such as GV arrest and oocyte degeneration, as well as necroptosis, have been found to have no detectable mutations. Conversely, some cases of POF/primary ovarian insufficiency have been associated with significant mutations, while others exhibit no detectable mutations at all. These findings have led to a re-evaluation of the spectrum of OMAs, and all forms of OMAs, including oocyte degeneration, EFS, ROS, and both classified and unclassified OMA, have been combined and classified as OMAs (8). Furthermore, Sang et al. (9) expanded the spectrum of OMAs in their mutation study by including zygotic cleavage failure and early embryonic arrest as additional components of OMAs.

Gulekli et al. (17) published a case report focusing on two women who had experienced MI arrest in their previous IVF cycles. In their clinical practice, they used unstimulated IVM as an alternative approach to address OMA but failed to achieve oocyte maturation. As a result, they concluded that the application of unstimulated IVM did not lead to significant outcomes for patients with OMAs (17). It is important to note that the cases included in their study specifically involved MI arrest, which is recognized as a particularly challenging subtype among OMAs cases.

This study aimed to evaluate the potential competence of oocytes from women with OMAs and to assess the role of ovarian stimulation in their development. We found that unstimulated IVM was only effective for patients with ROS. During the study, we made some valuable observations, including the observation of zona pellucida covering the oocytes of women with zona-free oocytes due to *TUBB8* mutation. We also found that in women with *TUBB8* mutation, we were unable to progress immature oocytes to MI and MII stages. Furthermore, in one woman with POF, we were not able to retrieve any oocytes both in unstimulated and letrozole primed IVM cycles. Although the results of this study do not provide any value for clinical use in OMAs, this study inspired the us to develop novel treatment options to overcome OMAs.

Study Limitations

The retrospective design and the small sample size are significant limitations of this study. The study team halted the study after realizing that unstimulated IVM was not effective in many cases, but this is the first study to examine the use of unstimulated IVM in women with different subtypes of OMAs. This experience has provided important insights into the limitations of unstimulated IVM for women with OMAs. Furthermore, this led the team to develop both physiological and supraphysiological IVM treatment modalities for women suffering from OMAs.

Conclusion

Unstimulated IVM does not appear to be an effective therapeutic option for women with OMAs. However, it is important to note that the study has limitations but it is stil the

first study to examine the use of unstimulated IVM in women with OMAs. Moreover, the study has provided the stimulus for us to develop of promising new treatment options.

Ethics Committee Approval: *The study received approval from the Ethical Committee of Medicana Samsun International Hospital (approval number: 7159, date: 27.12.2021).*

Informed Consent: *Written informed consent was obtained from all women with OMAs for all procedures.*

Author Contributions: *Surgical and Medical Practices: Ş.K., A.B., E.H., A.E.K., N.D.G., S.S.Ü., Y.C.Ü., Ş.H.; Concept: Ş.K., A.B., E.H., A.E.K., N.D.G., S.S.Ü., Y.C.Ü., Ş.H.; Design: Ş.K., A.B., E.H., A.E.K., N.D.G., S.S.Ü., Y.C.Ü., Ş.H.; Data Collection or Processing: Ş.K., A.B., E.H., A.E.K., N.D.G., S.S.Ü., Y.C.Ü., Ş.H.; Analysis or Interpretation: Ş.K., A.B., E.H., A.E.K., N.D.G., S.S.Ü., Y.C.Ü., Ş.H.; Literature Search: Ş.K., A.B., E.H., A.E.K., N.D.G., S.S.Ü., Y.C.Ü., Ş.H.; Writing: Ş.K., A.B., E.H., A.E.K., N.D.G., S.S.Ü., Y.C.Ü., Ş.H.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

Financial Disclosure: *The authors declared that this study received no financial support.*

References

1. Hatirnaz S, Hatirnaz E, Çelik S, Çalışkan CS, Tinelli A, Malvasi A, et al. Unraveling the Puzzle: Oocyte Maturation Abnormalities (OMAS). Diagnostics (Basel). 2022; 12: 2501.
2. Rudak E, Dor J, Kimchi M, Goldman B, Levran D, Mashiach S. Anomalies of human oocytes from infertile women undergoing treatment by in vitro fertilization. Fertil Steril. 1990; 54: 292-6.
3. Levran D, Farhi J, Nahum H, Glezerman M, Weissman A. Maturation arrest of human oocytes as a cause of infertility: case report. Hum Reprod. 2002; 17: 1604-9. Erratum in: Hum Reprod. 2002; 17: 2781.
4. Hourvitz A, Maman E, Brengauz M, Machtinger R, Dor J. In vitro maturation for patients with repeated in vitro fertilization failure due to "oocyte maturation abnormalities". Fertil Steril. 2010; 94: 496-501.
5. Beall S, Brenner C, Segars J. Oocyte maturation failure: a syndrome of bad eggs. Fertil Steril. 2010; 94: 2507-13.
6. Hatirnaz S, Başbuğ A, Hatirnaz E, Tannus S, Hatirnaz K, Bakay K, et al. Can in vitro maturation overcome cycles with repeated oocyte maturation arrest? A classification system for maturation arrest and a cohort study. Int J Gynaecol Obstet. 2021; 153: 496-502.
7. Galvão A, Segers I, Smitz J, Tournaye H, De Vos M. In vitro maturation (IVM) of oocytes in patients with resistant ovary syndrome and in patients with repeated deficient oocyte maturation. J Assist Reprod Genet. 2018; 35: 2161-71.
8. Hatırnaz Ş, Hatırnaz ES, Ellibeş Kaya A, Hatırnaz K, Soyer Çalışkan C, Sezer Ö, et al. Oocyte maturation abnormalities - A systematic review of the evidence and mechanisms in a rare but difficult to manage fertility pheneomina. Turk J Obstet Gynecol. 2022; 19: 60-80.
9. Sang Q, Zhou Z, Mu J, Wang L. Genetic factors as potential molecular markers of human oocyte and embryo quality. J Assist Reprod Genet. 2021; 38: 993-1002.
10. Dahan MH, Tan SL, Chung J, Son WY. Clinical definition paper on in vitro maturation of human oocytes. Hum Reprod. 2016; 31: 1383-6.
11. Hatırnaz S, Hatırnaz E, Dahan MH, Tan SL, Ozer A, Kanat-Pektas M, et al. Is elective single-embryo transfer a viable treatment policy in in vitro maturation cycles? Fertil Steril. 2016; 106: 1691-5.
12. Wang X, Song D, Mykytenko D, Kuang Y, Lv Q, Li B, et al. Novel mutations in genes encoding subcortical maternal complex proteins may cause human embryonic developmental arrest. Reprod Biomed Online. 2018; 36: 698-704.
13. Fadini R, Dal Canto MB, Mignini Renzini M, Brambillasca F, Comi R, Fumagalli D, et al. Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study. Reprod Biomed Online. 2009; 19: 343-51.
14. Rao GD, Tan SL. In vitro maturation of oocytes. Semin Reprod Med. 2005; 23: 242-7.
15. Wang AC, Zhang YS, Wang BS, Zhao XY, Wu FX, Zhai XH, et al. Mutation analysis of the TUBB8 gene in primary infertile women with arrest in oocyte maturation. Gynecol Endocrinol. 2018; 34: 900-4.
16. Feng R, Sang Q, Kuang Y, Sun X, Yan Z, Zhang S, et al. Mutations in TUBB8 and Human Oocyte Meiotic Arrest. N Engl J Med. 2016; 374: 223-32.
17. Gulekli B, Olgan S, Aydiner F. In vitro oocyte maturation from unstimulated cycles: does it offer a realistic chance to overcome the problem of repeated oocyte maturation arrest in IVF? Arch Gynecol Obstet. 2011; 283(Suppl 1): 133-4.