Laboratory Outcomes of Rescue In-vitro Maturation in Women with Polycystic Ovary Syndrome, Diminished and Normal Ovarian Reserve: A Single Center Study

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Background: In-vitro maturation(IVM) is utilized to avoid ovarian hyperstimulation syndrome and decrease the cost of IVF. However, there are different opinions regarding its utility. We evaluated outcomes of rescue IVM in polycystic ovary syndrome, diminished and normal ovarian reserve.
Methods: This retrospective cohort involves 615 immature oocytes retrieved from 221 IVF cycles. Outcomes of in-vitro matured oocytes were compared to sibling in-vivo mature oocytes. Association between stimulation an study trigger protocol were analyzed.
Results: Laboratory outcomes of Rescue-IVM(R-IVM) matured oocytes showed no statistically significant difference among groups. In-vivo mature oocytes showed a significantly higher fertilization rate and blastocyst rate ($p<0.0001$) compared to in-vitro matured oocytes. Progestin primed protocol and combination/dual trigger had significantly higher maturation rates.
Conclusion: Immature oocytes undergoing R-IVM can potentially undergo maturation, fertilization and even developed to blastocyst stage. However, given the low efficiency of development to blastocyst stage, higher power studies are needed to evaluate its practical use.

Key words: in vitro maturation, rescue in vitro maturation, laboratory outcomes, IVF-ICSI

Introduction

Assisted reproductive technologies (ART) have had a tremendous impact on fertility treatment around the world. This includes the developments such as in vitro fertilization (IVF) and in vitro maturation (IVM). In in-vitro fertilization, oocyte maturation occurs in the patient's ovary following controlled ovarian stimulation and trigger. Despite this, a considerable proportion of the oocytes retrieved during conventional IVF cycles may be immature.

In patients undergoing IVF, a trigger is administered to mimic the luteinizing hormone (LH) surge and induce the resumption of meiosis in the oocytes in order to retrieve mature metaphase II (M-II) oocytes after 36 hours. Without a trigger in IVF treatment, most oocytes retrieved will be immature at the germinal vesicle (GV) stage. A reproductive technique known as in-vitro maturation (IVM) allows for the in vitro development of mature oocytes from immature oocytes. These IVM oocytes can then be fertilized to produce viable embryos and live births. The advantages of IVM in human oocyte are: avoidance of side effects from ovarian stimulation, reduced cost of medications and careful monitoring during ovarian stimulation.[1]

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In vitro maturation provides a one-of-a-kind model for studying oocyte competence and follicle growth. In the absence of exogenous gonadotropins, the initial IVM approach involves collecting oocytes from antral follicles measuring 8–12 mm. The retrieved oocytes, which are still at the immature germinal vesicle (GV) stage, are then developed in vitro to the mature metaphase II (MII) stage.[2] Clinically, the concept of IVM has been expanded by facilitating in vitro maturation of immature oocyte following hCG administration as in conventional IVF called rescue in vitro maturation. This technique has the potential to enhance the outcome of immature oocytes. However, it is not yet commonly employed in clinics due to inadequate maturation rates, despite being widely tested in animal models. In that sense, the recovery of immature oocytes from conventional IVF has been an unresolved problem. In a literature review by Huiying, et al., they even suggested naming all immature oocytes retrieved from IVF as 'Medical Unusable Oocytes' as none of them will be used for subsequent treatment and will be eventually discarded in some clinics.[3]

In our institution, immature oocytes collected after a standard controlled in vitro stimulation protocol are routinely incubated in a standard culture medium for 24 hours and re-evaluated prior to discarding the oocyte. If the oocyte matures, it is then fertilized by intracytoplasmic sperm injection and is eventually cryopreserved once developed. As of today, there have been no published local studies on the significance of rescue in vitro maturation.

Motivated by the experience gained over years of clinical practice in IVM, the present study aimed to evaluate the laboratory outcomes of immature oocytes retrieved from women with polycystic ovary syndrome (PCOS), diminished ovarian reserve (DOR), and normal ovarian reserve (NOR). Pursuing this objective, immature oocytes retrieved from IVF-COS cycles will be analyzed to assess their ability to mature and be fertilized after a 24-hour incubation. We also determined the association between the maturation rate and the stimulation and trigger protocol used.

Significance of the Project

This is the first local study to determine the maturation, fertilization, cleavage and blastocyst rate after rescue in vitro maturation. This will help the center and clinicians realize the value of routinely incubating the immature oocytes and help them counsel patients. The comparison between three groups and the association between the trigger given were also analyzed. This may help clinicians choose the trigger to be used in patients who may benefit from it.

Literature Review

Epidemiology of In vitro Fertilization

A total of 301,316 IVF cycles were reported by the Society for Assisted Reproductive Technology (SART)[4] among its member facilities across the United States in 2020. Initially used just to treat tubal factor infertility, IVF is now employed in infertile couples, fertility preservation and preimplantation genetic testing.

Male factor (34.8%) is the most common reason for IVF in couples, followed by reduced ovarian reserve (29.5%) and multiple male and female factors (17.8%).[5] Surprisingly, female age, length of infertility, and previous pregnancy were discovered to be factors in IVF success.[6]

Oocyte Maturation

Oocyte maturation is one of the last stages of its development. It is characterized as a meiotic re-entry that takes place soon before ovulation. Oocytes are arrested in prophase I of meiosis within the ovary until follicle-stimulating hormone (FSH) and luteinizing hormone (LH) stimulate follicular growth and development, which subsequently initiates the resumption of meiosis up to metaphase II.[7] Oocytes are then kept in meiotic arrest once again until fertilization occurs, at which point meiosis is then completed. Sterols, steroids, growth factors, cyclic adenosine monophosphate (cAMP), and gap junctions are a few crucial intracellular, paracrine, and structural components that appears to interact intricately in the induction of oocyte maturation.[8]

Rescue In vitro Maturation

The term " in vitro maturation" (IVM) is most commonly used to describe the maturation of

immature cumulus-oocyte complexes (COCs) in culture from prophase I, through meiosis I to reach metaphase II (MII), after their retrieval from follicles that have not been exposed to a preovulatory trigger.[8] This was mostly established on animal research. However, in clinical human IVF, the term "IVM" is frequently used to describe the in vitro maturation of oocytes that have been removed from follicles at the immature stage as Germinal vesicle (GV) or metaphase I (MI) after being exposed to exogenous gonadotropins and/or hCG (also known as "follicle priming") to increase the likelihood of obtaining some mature oocytes.[9] Clinical IVM is commonly interchanged with rescue- IVM (R-IVM). R-IVM which was defined by Jie, et al. as the maturation in vitro of immature oocytes collected from the conventional IVF cycles.[3] In a study by Lee, et al, E-IVM produced 1.5 additional embryos for transfer in patients with low functional ovarian reserve increasing the number of embryos available for transfer.[10]

It should be highlighted that even if an immature oocyte uses IVM to advance to the MII stage completing its nuclear maturation, it does not guarantee that it has completed cytoplasmic maturation and has attained full developmental competence. The effective fertilization and subsequent development of a mature oocyte depends on the synchronization of nuclear and cytoplasmic maturation.[11]

Process of Fertilization

Fertilization results from the fusion of the oocyte and the spermatozoon, which initiates a series of critical events that lead to the development of the zygote.[12,13] Meiotic maturation of the oocyte and its ensuing activation by the spermatozoon are two different processes that are essential for normal fertilization.[13] In order to attach to and fuse with the egg plasma membrane in vivo, the capacitated sperm must break through the zona pellucida. The soluble cytosolic factor transported by the sperm, in turn, initiates a chain of events that leads to intracellular calcium oscillation via the phosphoinositide- specific phospholipase C, which activates the oocyte.

Meiotic maturation of oocyte involves competence of both the nucleus and cytoplasm. Nuclear maturation includes the extrusion of the first polar body, which is characteristic of MII oocytes. Cytoplasmic maturation, on the other hand, is poorly understood but is assumed to be heavily dependent on the cumulus-oocyte complex.[12,13,14]

Fusion of the two parental gametes to form a zygote is followed by two important processes: the prevention of polyspermy via cortical and zona reactions, and the completion of meiosis. Three hours after fertilization, the second polar body of the egg is released, leaving a haploid chromosome. This is complemented by the chromatin material derived from the sperm, which restores the diploid number of the fertilized egg. Each pronucleus derived from the male and female gamete migrates toward each other, forming a spindle, ready for the first cell division.

The general objective of this study was to determine the laboratory outcome of rescue in-vitro maturation in women with polycystic ovary syndrome, diminished and normal ovarian reserve. Specifically, it aimed to compare the maturation rate, fertilization rate, cleavage rate and blastocyst rate of immature oocytes in women with polycystic ovary syndrome, diminished and normal ovarian reserve. It also aims to determine and compare the maturation rate between the different stimulation protocol and triggers used. Lastly, to compare the fertilization rate and embryo growth of the rescue-IVM matured oocytes with their in vivo mature siblings.

Methods

This retrospective cohort study protocol was approved by Research and Biotechnology Division of St. Luke's Medical Center, Quezon City. A database generated from patients who underwent in vitro fertilization with intracytoplasmic sperm injection (IVF- ICSI) at the Center for Advanced Reproductive Medicine and Infertility (CARMI), St. Luke's Medical Center, Global City from January 1, 2020 to June 30, 2023 was retrospectively reviewed.

Inclusion and Exclusion Criteria

All oocytes retrieved from women who underwent oocyte retrieval at the Center for Advanced Reproductive Medicine and underwent rescue IVM were included in the study. Oocytes

retrieved from women who underwent IVF due to male factor and pelvic endometriosis were not included. Similarly, oocytes retrieved from patients with missing data were excluded in this study.

In Vitro Fertilization-Intracytoplasmic Sperm Injection (IVF-ICSI) Protocol

Ovarian Stimulation

Ovarian stimulation was performed using either GnRH antagonist, agonist or progestin primed protocol. Urinary or recombinant follicle stimulating hormone (FSH), with or without human menopausal gonadotropin (hMG) were started on the second or third day of the menstrual cycle. Pituitary suppression was achieved depending on the protocol used. When at least 1 follicle reached 18mm, or 2 follicles reached 17mm, ovulation trigger with either urinary or recombinant human chorionic gonadotropin, GnRH agonist or combination was administered.

Oocyte Retrieval

Oocyte retrieval under general or spinal anesthesia was scheduled 36 hours after the ovulation trigger. Ultrasound-guided oocyte retrieval was carried out using a transvaginal probe, attached to a gauge 16 double-lumen needle and connected to a collecting tube. Each follicle was punctured by the needle, and the follicular fluid was aspirated at 120 mmHg. The collecting tubes were immediately sent to the adjacent laboratory for evaluation for the presence of cumulus-oocyte complexes (COCs).

Oocyte Denudation

Oocyte denudation was done by treating the COCs with 10% hyaluronidase solution followed by mechanical removal of cumulus cells using a Pasteur pipette. This was within 1 hour, 1 hour to 2 hours and greater than 2 hours after the oocyte retrieval. Oocytes that were pre-incubated and were not immediately denuded were washed and transferred to culture dishes containing G-IVF medium and incubated under 6% CO_2 at 37°C. After denudation, the oocytes were examined in a microscope for maturational stage.

Rescue- In Vitro Maturation

Germinal vesicle or Metaphase I oocytes were incubated for 24 hours with standard culture conditions using Vitrolife G-IVF plus as the media. After incubation, growth of the oocyte was examined through a microscope by a certified embryologist.

Sperm Preparation

The semen specimen were collected by masturbation into a sterile container. The ejaculate was allowed to liquefy and pre-wash semen analysis was performed. Semen was prepared by 100%–70%–40% gradient system using SpermGrad (Vitrolife AB, Göteborg, Sweden). Semen specimen were placed on top of the discontinuous density gradient medium which was then centrifuged at 250 RPM for 15 minutes. G-IVF plus medium was added to the 100% fraction of pellet and then re-centrifuged for 5 minutes at 300 RPM. The final pellet was prepared via swim-up technique and the suspension was incubated under 6% CO_2 at 37°C, while preparing for ICSI.

Intracytoplasmic Sperm Injection (ICSI).

Intracytoplasmic sperm injection was performed on the heated stage of an inverted microscope under 400x magnification.[16] A single spermatozoon was selected and immobilized by compressing the tail of the sperm with an injection pipette. The immotile sperm was aspirated into the tip of the injection pipette. Oocyte was stabilized by an injection pipette with the polar body at the 6 or 12 o'clock position and was pierced by the injection pipette containing the immotile sperm at the 3 o'clock position[16]. After injection, oocytes were washed and incubated at 6% CO_2, 5% O_2 and 89% N2.

Assessment of Oocyte Survival and Fertilization

Injected oocytes were assessed for the presence of polar bodies and formation of two pronuclei (2PN) after 16 to 21 hours. Total fertilization failure was noted if none of the inseminated oocytes formed 2PN.

Data Collection and Analysis

The demographics collected includes female age, etiology of infertility, BMI, AMH, and age

of husband. The corresponding oocytes retrieved, matured, fertilized and embryo growth per group were likewise recorded.

Sample Size

The sample size was calculated based on the test of hypothesis for the difference in the maturation rate between hCG only and dual trigger (hCG and GnRH agonist). Assuming that maturation rate of trigger hCG is 70% and in dual trigger, 40%, (Yan etal, 2022), between alpha error of 5%, power of 95% and a one tailed alternative hypothesis, sample size calculated is 57 per group for a total of 228 for four groups. Controlling for 3 more variables in the analysis with an additional 25% for each control variable, final sample size required is 268.

Statistical Analysis

The maturation, fertilization, cleavage, and blastocyst rates were determined using frequency and percentages, while the comparisons between the three groups were analyzed using the chi square test. The comparison of the maturation rate between triggers and stimulation protocols, as well as the comparison between the fertilization, cleavage, and blastocyst rates of rescue IVM-matured oocytes to those of their in vivo mature siblings, were likewise analyzed using a chi square test with a significance level of alpha = 0.05.

Ethical Considerations

The study abided by the Principles of the Declaration of Helsinki (2013) and conducted along the Guidelines of the International Conference on Harmonization-Good Clinical Practice (ICH-GCP), E6 (R2) and other ICH-HCP 6 (as amended); National Ethical Guidelines For Health and Health-Related Research (NEG HHRR), 2017. The Clinical Protocol and all relevant documents were reviewed and approved by the SLMC Ethics Review Committee. Patient confidentiality was respected by ensuring anonymity of patient records. Each patient document was CODED and did not contain any identifying information in order to ensure confidentiality. All study data were recorded and investigators were responsible for the integrity of the data i.e accuracy, completeness, legibility, originality, timeliness and consistency. The manner of disseminating and communicating the study results guaranteed the protection of the confidentiality of patient's data. All study-related documents such as the all versions of the protocol, ethical clearance, data collection forms, hard copies of source documents shall be kept and stored by the Principal Investigator in strict confidentiality for at least 5 years' after which they will be shredded.

Results

Out of 1662 cycles performed in CARMI, SLMC-GC between January 2020 and June 2023, a total of 221 IVF-ICSI cycles were included and analyzed. The clinical characteristics across the 3 groups in this study are shown in Table 1.

In the 221 IVF-ICSI cycles there were a total of 615 immature oocytes where 310 of them were retrieved from women with polycystic ovary syndrome (PCOS), 81 with diminished ovarian reserve and 224 with normal ovarian reserve. Majority of the women were from the age of 35 to 40 years old (42.9%) while 30.1% were less than 35. The maternal BMI was likewise determined and showed that patients with a BMI of 18.5 to 22.9 kg/m accounted for 35.9% while 24.6% had BMI of 25 to 29.9 kg/m^2. The age and BMI were noted to be statistically different among the 3 groups. While the age of the husband was not statistically different, with an average age of 38.4 years old (SD = 13.7).

The baseline parameters across the various triggers used showed that maternal age, BMI, and etiology of infertility were significantly different among the four trigger groups. Meanwhile, the mean age of the husband is not significantly different. (Table 2)

A chi-square test was performed to analyze the existence of any association between the age and presence of immature oocytes and its competence. (Table 3) Results showed that immature oocytes are associated with maternal age, where the presence of GV is significantly increased in women less than 35 years old, while the presence of MI oocytes is higher with those 35 to 40 years old and >40 years old. Moreover, in-vitro matured oocytes significantly increased in age groups between 35 and 40 years old and >40 years old. Fertilization rate however did not differ significantly between the age groups.

Table 1. Baseline parameters between the 3 groups

	All Patients (n=615)		PCOS (n=310)		Diminished (n=81)		Normal (n=224)		p value
	n	%	n	%	n	%	n	%	
Age									
< 35	185	30.1	131	42.3	10	12.3	44	19.6	0.0001
≥35 - <40	264	42.9	133	42.9	34	42.0	97	43.3	
>40	166	27.0	46	14.8	37	45.7	83	37.1	
BMI (kg/m$^{2)}$									
<18.5	29	4.7	10	3.2	1	1.2	18	8.0	0.0001
8.5-22.9	221	35.9	108	34.8	26	32.1	87	38.8	
23-24.9	131	21.3	52	16.8	35	43.2	44	19.6	
25-29.9	151	24.6	81	26.1	15	18.5	55	24.6	
>30	83	13.5	59	19.0	4	4.9	20	8.9	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age of husband	38.4	13.7	37.7	18.5	38.3	4.5	39.3	5.8	0.396

Table 2. Baseline parameters between trigger used

	hCG (n=85)		Recombinant hCG (n=256)		GnRH (n=87)		Combination (n=187)		p-value
	n	%	n	%	n	%	n	%	
Age									
< 35	12	14.1	49	19.1	54	62.1	70	37.4	0.0001
≥35 - <40	53	62.4	112	43.8	19	21.8	80	42.8	
>40	20	23.5	95	37.1	14	16.1	37	19.8	
BMI									
<18.5 kg/m^2	0	0.0	4	1.6	7	8.0	18	9.6	0.0001
18.5-22.9 kg/m^2	14	16.5	97	37.9	22	25.3	88	47.1	
23-24.9 kg/m^2	38	44.7	46	18.0	20	23.0	27	14.4	
25-29.9 kg/m^2	27	31.8	58	22.7	20	23.0	46	24.6	
³30 kg/m^2	6	7.1	51	19.9	18	20.7	8	4.3	
Etiology of fertility									
Tubal	19	22.3	48	18.8	30	34.5	35	18.7	0.0027
Ovulatory	22	25.8	108	42.2	79	90.8	115	61.5	0.0001
Diminished	25	29.4	49	19.1	0	0.0	19	10.2	0.0001
Advanced maternal age	71	82	206	80.5	34	39.1	116	62.0	0.0001
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age of husband	38.1	4.6	39.9	20.3	36.8	4.3	37.2	5.2	0.124

Table 3. Maternal age and immature oocytes and competence

	<35		>35 to <40		>40		p value
	n	%	n	%	n	%	
GV (Germinal vesicle)	119	64.3	117	44.3	77	46.4	0.0001
MI (Metaphase I)	66	35.7	146	55.3	90	54.2	0.0001
Matured in vitro	39	21.1	93	35.2	57	34.3	0.003
Fertilized in vitro	17	43.6	42	45.2	30	52.6	0.5966

To evaluate the existence of any correlation between the laboratory outcomes and ovarian reserve a chi-square test was likewise done. It showed that the presence of germinal vesicle was higher in the PCOS group, while metaphase I oocytes were higher in the diminished ovarian reserve group. This however was not statistically significant ($p>0.05$). The authors also noted the maturation, fertilization, cleavage and blastocyst rates were not significantly different in the three groups ($p>0.05$). (Table 4) The overall maturation rate is 31% where 19% is from GV and 81% is from MI.

Upon comparison of maturation rate with the stimulation protocol used, it showed that the maturation rate was significantly increased in the progestin-primed group. (Table 5) On the other hand, when maturation rates were compared between the different triggers used, results showed that maturation rate was significantly increased in the combination of GnrHa and hCG (dual trigger) compared to the alone groups. (Table 6)

The sibling in-vivo matured oocytes of the three groups were analyzed and compared. Results revealed no significant difference between the fertilization and cleavage rates. While, the blastocyst rate was significantly low in diminished ovarian reserve than PCOS and normal ovarian reserve. (Table 7)

A comparison of the laboratory outcomes between the sibling oocytes in-vivo matured oocytes of each group showed that the outcomes of in vitro and in vivo matured oocytes in the DOR group are not statistically significant. (Table 9) In contrast, the sibling in-vivo matured oocytes of the PCOS and NOR group demonstrated statistically higher fertilization and blastocyst rates. (Table 8, Table 10)

Table 4. Comparison of laboratory outcomes of R-IVM in PCOS, diminished and normal ovarian reserve

	PCOS		Diminished ovarian reserve		Normal ovarian reserve		p-value
	n	%	n	%	n	%	
GV	168	(54.2)	33	(40.7)	112	(50.0)	0.0924
GV maturation	23	13.7	6	18	7	6.3	
M1	142	(45.8)	48	(59.3)	112	(50.0)	0.0924
M1 Maturation	73	51.4	25	52	55	49	
Maturation rate (GV + M1)	96	31.0	31	38.3	62	27.7	0.2068
Fertilization rate	41	42.7	16	51.6	32	51.6	0.4716
Cleavage rate	28	68.3	9	56.3	17	53.1	0.3881
Blastocyst rate	10	24.4	0	0.0	4	12.5	0.0621

Lastly, comparison of the laboratory outcomes between in-vitro matured and in-vivo matured oocytes showed that in vivo matured oocytes have a significantly higher fertilization rate and blastocyst rate, while there was no significant difference in the cleavage rate between the two groups. (Table 11)

Table 5. Maturation rate and COS protocol

	Antagonist		Agonist		Progestin Primed		p-value
	n	%	n	%	n	%	
Maturation rate	162	29.3	3	20.0	24	50.0	0.0079

Table 6. Maturation rate and trigger used

	hCG		Recombinant hCG		GnRHa		Combination		p-value
	n	%	n	%	n	%	n	%	
Maturation rate	29	34.1	79	30.9	14	16.1	67	35.8	0.0093

Table 7. Comparison of laboratory outcomes of in vivo matured oocytes in PCOS, diminished and normal ovarian reserve

	PCOS		Diminished ovarian reserve		Normal ovarian reserve		p-value
	n	%	n	%	n	%	
Fertilization rate	702	69.4%	79	65.8%	464	69.2%	0.7291
Cleavage rate	377	53.7%	52	65.8%	266	57.3%	0.086
Blastocyst rate	305	43.4%	10	12.7%	187	40.3%	0.0001

Table 8. Comparison of in vitro matured and in vivo mature oocytes in PCOS group

	R-IVM matured oocytes		In vivo mature oocytes		p value
	n	%	n	%	
Fertilization rate	41	42.7	702	69.4%	0.0001
Cleavage rate	28	68.3	377	53.7%	0.0684
Blastocyst rate	10	24.4	305	43.4%	0.0165

Table 9. Comparison of in vitro matured and in vivo mature oocytes in DOR group

	R-IVM matured oocytes		In vivo mature oocytes		P value
	n	%	n	%	
Fertilization rate	16	51.6	79	65.8%	0.1453
Cleavage rate	9	56.3	52	75.4%	0.1282
Blastocyst rate	0	0.0	10	12.7%	0.2037

Table 10. Comparison of in vitro matured and in vivo mature oocytes in NOR group

	R-IVM matured oocytes		In vivo mature oocytes		P value
	n	%	n	%	
Fertilization rate	32	51.6	464	69.2%	0.0048
Cleavage rate	17	53.1	266	57.3%	0.6426
Blastocyst rate	4	12.5	187	40.3%	0.0018

Table 11. Overall comparison of in vitro matured and in vivo mature oocytes

	R-IVM matured oocytes		In vivo mature oocytes		p value
	n	%	n	%	
Fertilization rate	89	47.1%	1245	69.1%	0.0001
Cleavage rate	54	60.7%	695	55.8%	0.3731
Blastocyst rate	14	15.7%	502	40.3%	0.0001

Discussion

As the woman ages, the number of follicles recruited into the developing follicle pool decreases. Most women over 40 suffer with diminished ovarian reserve. Furthermore, some studies showed that a higher proportion of immature oocytes in patients with diminished ovarian reserve are observed compared to those with normal ovarian reserve.[10] These women continuous to be a therapeutic challenge in all fertility facilities, regardless of the underlying causes.[10] In a study by Lee, et al, they demonstrated a 60% improvement in the number of available embryos for transfer, hence, recommend the use of R-IVM to improve IVF outcomes in such patients.[10]

The use of rescue IVM was investigated due to increase number of immature oocytes retrieved in an IVF cycle. Based on the literature review by Huiying et al., immature oocytes were seen to account for 15–30% of the total number of retrieved oocytes in a conventional IVF cycle.[3] In this study, a total of 615 immature oocytes were retrieved from a total of 2418 oocytes, accounting for 25.4%, which was within range from international literature. The presence of germinal vesicle was noted to be higher in the PCOS group, while metaphase I oocytes were higher in the diminished ovarian reserve group. This however was not statistically significant. Increase in immature oocytes in diminished ovarian reserve and PCOS may indicate asynchronous follicle maturation and or the inability to properly mature in response to gonadotropin stimulation.[10]

The reproductive potential of in vitro-matured oocytes remains unknow.[16,17] However, in women where mature oocytes retrieved in vivo are low after a conventional IVF cycle, every additional oocyte and embryo will increase the chance of conception. Because of this, rescue IVM is done in our center as a strategy for increasing cycle outcomes, especially for patients with poor oocyte yield upon retrieval. To our knowledge, this is the first local study to evaluate laboratory outcomes of rescue in vitro maturation and the first study comparing laboratory outcomes of immature oocytes between PCOS, diminished, and normal ovarian reserve.

In a study by Lee, et al., they compared low functional ovarian reserve with normal functional ovarian reserve. Their result showed the maturation rate (75.5% vs. 78.3%), fertilization rate (59.5% vs. 67.7%), and development rate (72.7 vs. 86.4%) were similar in both groups.[10] Similarly, in this study, the maturation, fertilization, cleavage and blastocyst rates were not significantly different in the three groups ($p>0.05$). In comparison, our laboratory outcomes are notably lower than those stated in

the study. Variations in our laboratory outcomes may be secondary to the different duration of incubation, protocols and triggers used compared to the previous study, where they incubated the immature oocytes up to 48 hours and used only a GnRH long agonist protocol and hCG as the trigger. Likewise, developmental rates, specifically the low blastocyst rate, may be attributed to the discretion of the attending physician to freeze embryos at Day 3.

Controlled ovarian stimulation (COS) is the core of most assisted reproductive technologies (ART). Different COS protocols are employed during ART depending on the patient and attending physician. Comparisons of various protocols have been done; however, there are still no studies on the oocyte outcome of these protocols in rescue in vitro maturation. In this study, we determined the maturation rate across the three protocols used. Results showed that the maturation rate was significantly increased in the progestin- primed group. In a previous study by Lee et al., they only used the long GnRHa protocol, and they had higher outcomes, although they had a significantly smaller sample size.[10]

Oocyte maturation in in-vitro fertilization cycles is typically triggered by hCG which is used as a substitute for a normal LH surge. Despite proper hCG administration, some retrieved oocytes are arrested at the germinal vesicle or metaphase I stage. Following the introduction of the GnRH antagonist protocol, the practice of using a gonadotropin-releasing hormone agonist (GnRH-a) to elicit an endogenous LH surge has been investigated in numerous clinical situations, especially for preventing ovarian hyperstimulation syndrome. Among the benefits of utilizing GnRH-a to induce oocyte maturation is the activation of a more physiological secretion of LH and follicle-stimulating hormone (FSH), which resembles a regular menstrual cycle.[18] Some studies imply an increase in the percentage of mature oocytes recovered when stimulated with GnRH-a compared to hCG.[19,20] Shapiro et al. developed the concept of ''dual trigger'' using a combination of GnRH-a and low-dose hCG.[21] Dual trigger then has been studied to improve oocyte maturity rates in a conventional IVF. In this study, we compared the maturation rate among the triggers used to determine which might help improve the maturation rate of immature oocytes. Our results showed a significantly increased maturation rate in the combination of GnrHa and hCG compared to the alone groups, which is similar to that of the previously stated study. This finding is supported by a study by Griffin, et al., where they noted an increase in the percentage of mature oocytes after the use of a dual trigger with a GnRH agonist and hCG in patients with a previous cycle with more than 25% of immature oocytes retrieved.[18] Also, in a recent study by Albeitawi, et al, where they compared dual trigger with hCG only trigger in normal responders, they noted higher oocyte yield, mature oocytes and fertilized oocytes in the dual trigger group.[22]

Laboratory outcomes of rescue-IVM matured oocytes have been compared to those of their MII sibling oocytes. Our results showed that the fertilization rate of R-IVM matured oocytes was lower than that of their MII sibling oocytes, which is consistent with previous reports.[23,24,25] On the other hand several other studies reported comparable fertilization rates.[14,26,27,28] Variation in fertilization rate and subsequent embryo growth outcomes may be explained by differences in the population, stimulation protocols, culture condition, and duration.[17]

The developmental competence of each group was also determined and analyzed. Results showed that the cleavage rates between the 2 groups were comparable (60.7% vs 55.8%), while the blastocyst rate was significantly decreased in R-IVM-matured oocytes. This is in contrast to the study by Andi et al. where their data showed a significantly higher rate of cleavage arrest in R-IVM matured oocytes compared to their in vivo mature sibling.[17] A study by Escrich et al. showed a similar blastocyst rate between the 2 groups (44% vs. 65%), while other recent studies showed a lower blastocyst rate, which is similar to this study with only 15.7%.[29,30,31] Data on blastocyst development from R-IVM matured oocytes are scarce, as earlier studies reported on the culture of embryos up to day 3 development only.[17] Similarly, the lower blastocyst rate and higher cleavage rate among the diminished ovarian reserve group are likely due to the decision of the attending physician to cryopreserve embryos that reached Day 3.

Different rates of fertilization (40–90%) and embryo development (25–98%) of immature oocytes

obtained after stimulation have been shown in earlier investigations.[10] The discrepancies are likely due to various techniques used in oocyte and embryo culture, as well as the timing of intracytoplasmic sperm injection (ICSI) in relation to culture initiation. Longer duration of IVM culture (12-48 h) after oocyte retrieval might yield improved clinical outcomes.[10]

Conclusion

The overall maturation rate is 31% where approximately half of MI oocytes mature to MII, but less than a fifth of GV oocytes mature in any of the three categories. The maturation, fertilization, cleavage and blastocyst rates were not significantly different in the three groups. In vitro matured oocytes have a lower blastocyst rate than in vivo matured oocytes in all groups. The fertilization rate of R-IVM matured oocytes was lower than that of their MII sibling oocytes, while cleavage rates were comparable. The blastocyst rate was significantly decreased in R-IVM-matured oocytes.

In general, the in vitro matured oocytes have lower laboratory outcomes. The DOR group is unsuitable for rescue- IVM; even the in vivo-developed oocytes have a poor outcome. Nonetheless, immature oocytes that undergo rescue IVM can still potentially undergo maturation, fertilization and even developed to blastocyst stage. However, given the low efficiency of development to blastocyst stage, the use of rescue IVM is currently not recommended.

The dual trigger and progestin-primed stimulation protocol is associated with an increased maturation rate in rescue in vitro maturation.

Recommendations

Higher power studies are needed to evaluate the practical use of R-IVM to be able understand its utility in routine laboratory practice or in low-resource settings. Studies to examine the role culture media and duration of incubation is also of importance. Likewise, since this is the first study to investigate the probable impact of the trigger used on the outcome of rescue in vitro maturation, future research to validate the findings may be conducted.

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