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The potential application of conditioned media-mesenchymal stem cells on human oocyte maturation in assisted reproductive technology: A quasi-experimental based-study at Dr. Sardjito General Hospital, Yogyakarta, Indonesia

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ABSTRACT

Background: PCOS typically characterized by a decreased level of GDF9 and BMP15, which contribute to follicular arrest. Recent research related to stem cells showed that conditioned media-mesenchymal stem cell (CM-MSC) might have a positive role in folliculogenesis. Therefore, this study aimed to assess the effectiveness of CM-MSC towards oocyte maturity Method: A quasi-experimental design was used to examine the outcome of 3 different interventions, namely group A (4-hour incubation in standard media), group B (24-hour incubation in standard media), and group C (24 hours incubation in conventional media with addition of CM-MSC). Nuclear and cytoplasmic maturity, along with GDF9 and BMP15 levels were measured and analyzed.
Results: Sixty-three patients at an infertility clinic, RSUP Dr. Sardjito, Yogyakarta was recruited and a total of 292 germinal vesicles were obtained to start *in vitro* maturation procedure. Multivariate analysis showed group C has OR 6.9 (3,3–14,41) to obtain metaphase II oocyte than group B (p <0,0001). Infertility causes, insulin resistance, and maternal age risk are other factors that significantly influence oocyte maturity outcome (p <0.001; p = 0.005; p = 0.017). For the oocyte morphology outcome, no significant effect was obtained from the intervention (p> 0.05). Group C has higher GDF9 levels (Δ mean = 3.31) and BMP15 (Δ mean = 1.52) compared with group B (p <0.001; p = 0.006).
Conclusion: It can be concluded that 24-hour incubation in CM-MSC was effective to induce oocyte maturation. However, other factors, such as infertility causes, insulin resistance, and maternal age, should also be considered.

Keywords: In vitro maturation; Conditioned Media-Mesenchymal Stem Cells; Oocyte Maturation; BMP15; GDF9
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INTRODUCTION

Assisted Reproductive Technology (ART) is an essential solution for infertility problems experienced by 10-15% of couples in Indonesia with an estimated 200,000 couples will consult to infertility clinics every year.[1] The GnRH antagonist protocol is the most globally used in ART clinics around the world to stimulate the ovaries, but there is wide variability within the outcome of this technique. In addition, specific subgroup, such as polycystic ovary syndrome (PCOS) patients are often considered as challenging cases in which obtaining mature oocyte is very difficult.[2,3]

DeVos, et al.[4] reported a 5.3% implantation rate in pregnancy obtained from the results of rescued in vitro maturation (IVM) which assists oocyte maturation prior to fertilization procedure. Furthermore, in the group of patients with low functional ovarian reserve, rescued IVM maturation of immature oocytes was reported to provide 60% increase of mature oocytes.[5]

However, an alternative and more efficient procedure are needed to be developed in order to enhance the success rate of assisted reproduction. One of the promising methods is by utilizing mesenchymal stem cells combined with conditioned media (CM-MSC). This method was developed based on the theory of the positive impact of paracrine effect of stem cells toward folliculogenesis, which includes trophic effects (anti-apoptosis, mitotic stimulation, and proliferation), immunomodulators, angiogenesis, anti-scarring effect, chemoattractants, inducing differentiation and sometimes fusing with target cells. All of these effects have the potential to enhance the oocyte maturation.[6-8]

In evaluating the efficacy of CM-MSC toward oocyte maturity, it is necessary to assess the nuclear maturity aspects (presence of Polar Body I), cytoplasmic maturity (spindle metaphase plate configuration and mitochondrial activity), and level of oocyte-secreted factors (OSF), specifically GDF9

and BMP15, which both are known to regulate granulosa and cumulus cell function.[4,9,10] However, scientific evidence of CM-MSC is required to support its implementation as routine oocyte maturation procedure in assisted reproductive technology. Therefore, this study aimed to determine whether the addition of CM-MSC is valuable as *in vitro* oocyte maturating agent among infertile patients, including those with polycystic ovary syndrome.

METHODS

Study Design

A laboratory-based quasi-experimental study was conducted to evaluate CM-MSC on oocyte maturity outcome as assessed by the presence of polar body I morphology, mitochondrial activity, spindle configuration, GDF9, and BMP15 levels. This research was conducted after obtaining an Ethical Clearance from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Gadjah Mada University with ethical clearance number KE/FK/1011/EC/2018

Subject

A total of 292 germinal vesicles oocytes were obtained as study samples from 63 maternal subjects at Permata Hati Infertility Clinic, Dr. Sardjito General Hospital during 2017. Samples were obtained after detailed informed consents were delivered to the donors. The inclusion criteria was immature oocytes collected from the OPU (Ovum Pick Up) procedure for IVF patients at the Permata Hati infertility clinic. Immature oocytes are defined as GV (Germinal Vesicle) in this study. Exclusion criteria were immature oocytes with the abnormal vacuole, abnormalities of the zona pellucida, giant oocyte, and abnormalities of the cytoplasmic form.

Conditioned Media - Mesenchymal Stem Cell (CM-MSC)

CM-MSC fluid is derived from amniotic membrane mesenchymal cells cultures and stored in refrigerator at -20°C before use. Initial pre-warmed and equilibrated in an incubator (37°C; 5% O2; 6% CO2) were needed before the medium could be used.

Immature oocytes are obtained from OPU procedure. Two to six hours after the OPU procedure, OCC (oocyte cumulus complex) will be denuded to determine the maturation stages of the oocyte. Denudation was performed by incubating OCC in hyaluronidase / Hyas (Vitrolife, Sweden) as the standard media solution, followed by mechanical separaton of cumulus cells using a denudation pipette with a diameter of 170 and 140 µm (Humagen, Origio, Denmark). Due to ethical considerations and previous consent from patients, only GV oocytes were allocated into 3 different group of intervention, namely: group A (4 hour incubation in standard media), group B (24-hour incubation in the standard media), and group C (incubation for 24 hours in standard media with the addition of 50% CM-MSC).

The determination of nuclear maturity was conducted using 32× magnification inverted microscope (Olympus 71) started after incubation to observe the emergence of first polar body during second meiosis (MII: metaphase II) which reflects complete oocyte maturity. Cytoplasmic maturity of oocyte was evaluated by examining cytoplasmic mitochondrial activity using MitoTracker® Red 580 FM M22425 (Invitrogen, Eugene, Oregon, USA). Mitochondrial activity was observed by the fluorescent microscope and positive result was marked by red fluorescence. The intensity and area of fluorescence were then calculated using the ImageJ application. After evaluating the nuclear and cytoplasm maturation, GDF9 and BMP15 expression were measured using qRT-PCR using 0.1 ml supernatant.

RESULT

Subjects Baseline Characteristics

Sixty-three patients from Permata Hati Infertility Clinic in Dr. Sardjito General Hospital who fulfilled the inclusion criteria and did not meet the exclusion criteria were recruited as the study sample. After being given a stimulation protocol, the total OCC collected from 63 patients was 791 in total, with 179 OCCs obtained from PCO syndrome patients, 287 OCCs obtained from PCO patients without syndromes and 425 OCCs obtained from patients with tubal disease. Oocytes in the GV phase will be allocated into three different interventions group (A, B, and C) and subsequent description and analysis of the sample will assume one GV as one individual sample with a total of 292 GV samples.

As shown in Table 1, there are heterogeneities of the samples based on the age groups, insulin resistance category, and the estradiol level (P <0,005). The heterogeneities will be controlled by using multivariate analysis if those variables significantly influence oocyte maturity.

Bivariate Analysis among Factors Influencing the Oocyte Maturation Outcomes

According to table 2, there are significant influences from the intervention, cause of infertility, category of insulin resistance, and category for maternal age on

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Table 1 Baseline Characteristics of Subjects Based on Intervention

Total Sampel GV n = 292	Group A n = 40 (13.7%)	Group B n = 84 (28.8%)	Group C n = 168 (57.5%)	P-Value
Maternal Age Group				
• Low Risk (24-35)	21 (52.5%)	70 (83.3%)	121 (72.0%)	*0.001
• High Risk (<24->35)	19 (47.5%)	14 (16.7%)	47 (28.0%)	
Maternal Body Mass Index				
• Low Risk (18,5–24,9)	17 (42.5%)	37(44.1%)	84 (50.0%)	0.544
• High Risk (≥25)	23 (57.5%)	47(55.9%)	84 (50.0%)	
Insulin Resistance				
• Normal	30 (75.0%)	28 (33.3%)	79 (47.0%)	** <0.001
• Insulin Resistant	10 (25.0%)	56 (66.7%)	89 (53.0%)	
Cause of Infertility				
• PCO with syndrome (%)	14 (35.0%)	26 (31.0%)	43 (25.6%)	0.393
• PCO without syndrome (%)	9 (22.5%)	21 (25.0%)	58 (34.5%)	
• Tubal disease (%)	17 (42.5%)	37 (44.0%)	67 (39.9%)	
AMH Median (range)	5.51 (2.01-8.87)	4.15 (0.37-12.88)	4.25 (1.02-13.24)	0.270
FSH Median (range)	2025 (1075-5555)	1850 (1250-2700)	2000 (750-3450)	0.798
E2 Median (range)	2155 (533-6276)	3242,50 (574 – 9253)	3211 (424 – 5618)	***<0.001

Group A (4-hour incubation in Conditioned Media only); Group B (24-hour incubation in Conditioned Media without Mesenchymal Stem Cells); Group C (24-hour incubation with Conditioned Media and Mesenchymal Stem Cells); GV (*Germinal Vesicle*)
* p-value between group A vs group B (<0.001); group A vs group C (0.02); dan group B vs group C (0.05)
** p-value between group A vs group B (<0.001); group A vs group C (0.001); dan group B vs group C (0.038)
*** p-value between group A vs group B (<0.001); group A vs group C (<0.001); dan group B vs group C (0.261)

Table 2 Bivariate Analysis among Factors Influencing the Oocyte Maturation Outcomes

		Metaphase II			
***Crude* PR**	**Nuclear Maturation Metaphase II**	**Fine Polar Body**	**Fine Zona Pellucida**	**Fine Cytoplasm**	**Normal Distribution of Active Mitochondria**
Intervention					
• Group B	***ref***	*ref*	*ref*	*ref*	*ref*
• Group C	1.68 (1.27 – 2.22) *P <0.0001	1.44 (0.65 – 3.18) P = 0.35	1.11 (0.76 – 1.61) P = 0.59	1.19 (0.84 – 1.69) P = 0.28	1.24 (0.91 – 1.68) P = 0.12
• Group A	N/A	N/A	N/A	N/A	N/A
Cause of Infertility					
• Tubal disease	***ref***	*ref*	*ref*	*ref*	*ref*
• PCO without Syndrome	0.66 (0.52 – 0.85) P <0.001	0.89 (0.45 – 1.78) P = 0.73	0.82 (0.58 – 1.19) P = 0.29	0.73 (0.51 – 1.03) P = 0.052	0.97 (0.76 – 1.23) P = 0.80
• PCO with Syndrome (PCOS)	0.32 (0.21 – 0.48) *P <0.0001	1.09 (0.47 – 2.52) P = 0.84	0.92 (0.58 – 1.46) P = 0.70	1,04 (0.74 – 1.47) P = 0.81	0.89 (0.61 – 1.28) P = 0.48
Resistence of Insulin					
• Normal	***ref***	*ref*	*ref*	*ref*	*ref*
• Insulin Resistant	0.71 (0.57 – 0.89) *P <0.01	0,73 (0,40 – 1,34) P = 0.31	0.87 (0.64 – 1.18) P = 0.37	0.83 (0.63 – 1.08) P = 0.16	0.89 (0.71 – 1.11) P = 0.29
Maternal Age Group					
• Low Risk (24-35)	ref	ref	ref	ref	ref
• High Risk (<24 or >35)	0.54 (0.38 – 0.76) *P <0.001	0.82 (0.35 – 1.91) P =0.638	1.04 (0.71 – 1.53) P = 0.83	1.06 (0.77 – 1.47) P = 0.72	1.34 (1.1 – 1.62) *P = 0.028

Table 2 *Continue*

Crude PR	Nuclear Maturation Metaphase II	Metaphase II			
		Fine Polar Body	Fine Zona Pellucida	Fine Cytoplasm	Normal Distribution of Active Mitochondria
Maternal Body Mass Index	ref	ref	ref	ref	ref
• Low Risk (18,5-24,9)	0.92 (0.73 – 1.15)	1.46 (0.81 – 2.65)	0.97 (0,72 – 1.31)	1.11 (0.86 – 1.44)	0.99 (0.80 – 1.23)
• High Risk (≥25)	P =0.474	P = 0.207	P = 0.858	P = 0.42	P = 0.94

Group A (4-hour incubation in Conditioned Media only); Group B (24-hour incubation in Conditioned Media without Mesenchymal Stem Cells); Group C (24-hour incubation with Conditioned Media and Mesenchymal Stem Cells); GV (Germinal Vesicle); ref (Reference Category); N/A (not applicable : cannot be calculated due to any zero values in the group)
* p-values are statistically significant (p < 0.05)

Table 3 **Bivariate Analysis of Hormonal Level to Oocyte Maturation Outcome**

OR P-value	Nuclear Maturation Metaphase II	Fine Polar Body	Fine Zona Pellucida	Fine Cytoplasm	Normal Distribution of Active Mitochondria
AMH	0.88 (0.79 – 0.97) *0.013	1.02 (0.85 – 1.22) 0.84	0.86 (0.76 – 0.96) *0.008	0.90 (0.81 – 1.00) 0.05	0.98 (0.89 – 1.09) 0.71
FSH	1.00 (1.00 – 1.00) 0.911	1.00 (1.00 – 1.00) 0.06	1.00 (0.99 – 1.00) 0,59	1.00 (0.99 – 1.00) 0.73	1.00 (0.99 – 1.00) 0.55
E2	1.00 (1.00 – 1.00) 0.96	1.00 (1.00 – 1.00) 0.36	1.00 (1.00 – 1.00) 0.17	1.00 (1.00 – 1.00) 0.52	1.00 (1.00 – 1.00) *0.009

* p-values are statistically significant (p < 0.05)

Table 4 **Mean Differences in Oocyte Secreting Factor**

Parameter	GDF9		BMP15	
	Mean ± SD	Mean Difference (CI 95%)	Mean ± SD	Mean Difference CI 95%
Intervention				
• Group B	29.32 ± 5.24	Ref	8.84 ± 4.12	Ref
• Group C	32.63 ± 4.19	3.31 (2.01 – 4.61) *P<0.001	10.37 ± 4.06	1.52 (0.44 – 2.61) *P= 0.006
Cause of Infertility				
• Tubal Disease	32.7 ± 4.99	Ref	10.62 ± 4.15	Ref
• PCO without syndrome	29.77 ± 5.24	-2.93 (-4.58 to -1.28) *P <0.001	8.94 ± 4.45	-1.68 (-3.06 to -0.30) *P= 0.011
• PCOS	27.67 ± 4.33	-5.03 (-6.71 to -3.35) *P <0.001	6.98 ± 3.54	-3.65 (-5.05 to -2.24) *P <0.001
Insulin Resistance				
• Normal	31.28 ± 5.52	Ref	9.56 ± 4.38	Ref
• Insulin Resistant	29.59 ± 5.0	-1.69 (-2.91 to -0.47) *P= 0.007	8.66 ± 4.27	-0.9 (-1.89 to 0.09) P= 0.077
Maternal Age Group				
• Low Risk (24-35)	31.15 ± 5.12	Ref	9.64 ± 4.28	Ref
• High Risk (<24 or >35)	28.38 ± 5.31	-2.77 (-4.1 to -1.43) *P<0.001	7.58 ± 4.15	-2.06 (-3.16 to -0.97) *P<0.001
Maternal Body Mass Index				
• Normal (18.5-24.9)	30.56 ± 5.28	Ref	9.43 ± 4.53	Ref
• Abnormal (≥25)	30.23 ± 5.35	-0.34 (-1.56 to 0.89) P= 0.591	8.76 ± 4.15	-0.67 (-1.68 to 0.33) P= 0.19

Group A (4-hour incubation in Conditioned Media only); Group B (24-hour incubation in Conditioned Media without Mesenchymal Stem Cells); Group C (24-hour incubation with Conditioned Media and Mesenchymal Stem Cells); GV (Germinal Vesicle); ref (Reference Category);
* p-values are statistically significant (p < 0.05)

Table 5 Multivariate Analysis of Factors Affecting Maturation Outcomes

Parameter	Nuclear Maturity Metaphase II	
	Crude OR (CI 95%)	*Adjusted* OR (CI 95%)
Intervention		
• Group B	*ref*	*ref*
• Group C	3.11 (1.8– 5.34) * P <0.0001	6.9 (3.3– 14.41) *P <0.0001
Cause of Infertility		
• Tubal disease	*ref*	*ref*
• PCO without syndrome	0.36 (0.20 – 0.64) *P <0.001	0.06 (0.02 – 0.18) *P <0.001
• PCOS	0.12 (0.61 – 0.22) *P <0.001	0.02 (0.004 – 0.06) *P <0.001
Resistance of Insulin		
• Normal	*ref*	*ref*
• Insulin Resistant	0.5 (0.31 – 0.79) * P = 0.003	4.77 (1.62 – 14.03) *P = 0.005
Maternal Age		
• Low Risk (24-35)	*ref*	*ref*
• High Risk (<24 or >35)	0.38 (0.21– 0.69) * P = 0.001	0.41 (0.19 - 0.85) *P = 0.017
AMH Level	0.88 (0.79 – 0.97) * P = 0.013	*Insignificant in Multivariate Model (p = 0.798)*

Group B (24-hour incubation in Conditioned Media without Mesenchymal Stem Cells); Group C (24-hour incubation with Conditioned Media and Mesenchymal Stem Cells); OR (odd ratio); ref (Reference Category);
* p-values are statistically significant (p < 0.05)

Table 6 Beta Coefficients for Oocyte Metaphase II Outcome

Parameter	Beta Coefficients	P-Value
Constant	0.574	0.079
Group C intervention	1.937	<0.001
Insulin Resistant	1.562	0.005
High Risk Maternal Age	- 0.905	0.017
PCO without syndrome	-2.864	<0.001
PCOS	-4.141	<0.001

pseudo R-squared (Nagelkerke) : 0.422

the outcome of oocyte maturation to achieve metaphase II. Regarding of mature oocytes morphology, no significant influence was observed from intervention, cause of infertility, insulin resistance, maternal age, and maternal BMI according to criteria such as fine polar bodies, smooth zona pellucida, smooth cytoplasm, and normally-distributed of active mitochondria (p> 0.05), with the exception of maternal age category which influenced active mitochondrial distribution (p = 0.028).

Group C intervention had 1.68 times higher prevalence of metaphase II oocytes compared to group B (p < 0,0001). The condition of PCO without syndrome has 0.66 times lower prevalence of metaphase II maturation than the tubal disease group, which represents the population without any ovarian abnormalities (p <0,0001). The condition of PCO with syndrome (PCOS) has 0.32 times lower prevalence of metaphase II maturation than the tubal disease group (p <0,0001). Those with insulin resistance had 0.71 times lower prevalence of oocyte that reaches metaphase II compared with the normal group (p <0.01). The high-risk maternal group had 0.54 times lower prevalence of oocyte that reaches metaphase II compared with the low risk group (p <0.001). The high-risk maternal age group also had 1.34 times higher prevalence of normally-distributed active mitochondria than the low risk group (p = 0.028).

According to Table 3, there is a significant effect of AMH level on nuclear maturity and fine zona pellucida. An increase in AMH level resulted in reduced odd of mature oocytes outcome with OR 0.88 (0.79 - 0.97) compared with the AMH levels one unit below (p = 0.013). Furthermore, elevation of AMH also decreased the odd of fine zona pellucida with OR 0.86 (0.76 - 0.96) compared with the AMH level one unit below (p = 0.008).

The table above (Table 4) shows mean differences in expression between GDF9 and BMP15 for each variables group. As shown in figure 1 and table 4, group C intervention has a significantly higher mean of GDF9 and BMP 15 expression at 3.31 and 1.52 respectively compared to group B (p <0.001; p = 0.006).

In the aspect of infertile etiology, a significant difference of GDF9 and BMP15 expressions were also observed. The PCO without syndrome group tend to had significantly lower mean expressions of GDF9 and BMP 15 by 2.93 and 1.68, respectively, compared to tubal abnormality group which represents population without ovarian abnormalities (p <0.001; p = 0.011). Moreover, the PCOS group tends to have significantly lower mean GDF9 and BMP15 expression by 5.03 and 3.65, respectively, compared to tubal disease group (p <0.001).

The same finding also observed when comparing those groups according to insulin resistance condition but only on the average expression of GDF9. Insulin resistant group had significantly lower GDF9 expression by 1.69 compared to normal group (p = 0.007). Maternal age also affects GDF9 and BMP15 expressions with high-risk

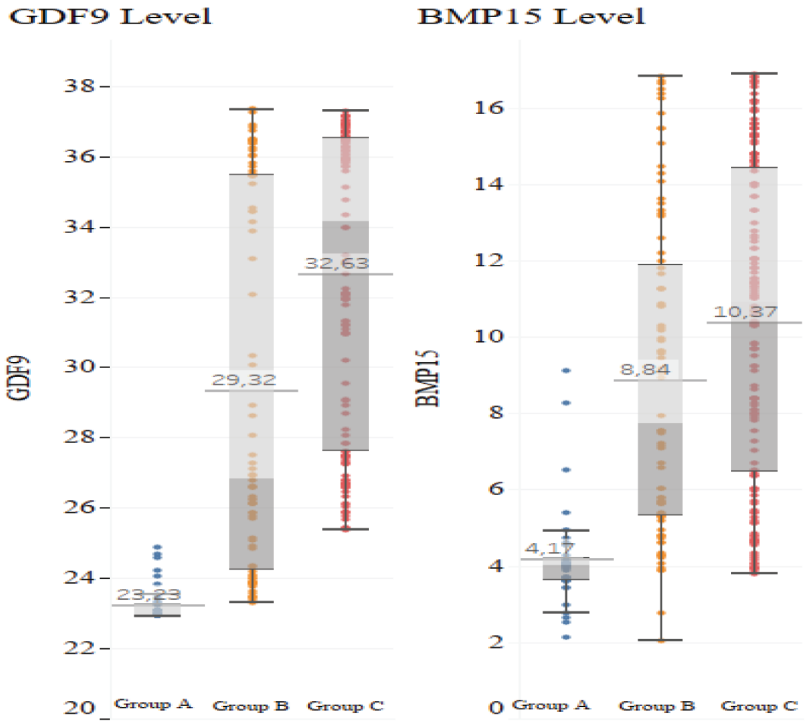

Figure 1 Mean Level of Oocyte Secreted Factors Based on Intervention Groups

maternal age had significantly lower GDF9 and BMP15 mean expression by 2.77 and 2.06, respectively compared to the category maternal risk is low (p <0.001).

Multivariate Analysis of Factors Affecting Oocytes Maturation

Multivariate analysis (table 5) confirmed that intervention, cause of infertility, insulin resistance, and maternal age risk category as the four main factors that had significant effects on oocyte maturity to attain metaphase II. According to the analysis, Group C intervention had odd 6.9 (3,3–14,41) times higher to obtain metaphase II nuclear maturity compared to group B (p <0,0001). In addition, PCO without syndrome condition had odd 0.06 (0,02 – 0,18) times lower than the tubal abnormalities to obtain oocyte metaphase II(p <0.001) while PCO with syndrome (PCOS) appear to had odd 0.02 (0,004 – 0,06) times lower compared to the tubal abnormalities (p <0.001). Furthermore, this relationship shows the linearity of the infertile etiology to attain favorable oocyte maturity, which the best odd was in the tubal abnormalities, followed by PCO without syndromes, and PCOS as the worst.

The condition of insulin resistance had odd of 4.77 (1,62 – 14,03) times higher to obtain metaphase II maturity compared to normal group (p = 0.005). The multivariate analysis result is contrasted with the bivariate analysis, which previously predicted that insulin resistant group as a risk factor for poor outcome of maturity.

According to on the model presented in Table 6, to get the best odds of having oocyte metaphase II, the group C intervention should be given into a candidate with insulin resistance with low-risk age group and those who do not have either PCO without syndrome or PCO with syndrome as the etiology of infertility.

DISCUSSION

ART is considered as the primary and most effective solution to date for couple with infertility.[1,2,3] However, even it still has low rate of implantation as reported by De Vos et.al.[4] Some studies has explored some methods to enhance oocyte maturity and, thus, fertilization and implantation rate.[11,12,13,14] One of those methods is the application of conditioned media from stem cells, particularly MSC.

Our finding provide one of the first evidences about the efficacy of CM-MSC in assisting human oocyte maturation. It appeared that 24-hours treatments is the most effective incubation period in order to enhance maturation rate, marked by increasing expression of GDF9 and BMP15. However, it was also apparent that this result could be influenced by several factors that could also influence maturation process. In addition, our model predict that CM-MSC work best on oocytes that obtained from individual without PCO or PCOS which hinted that those who has these condition could still posed a challenge for a gynecologists.

The usage of stem cell conditioned media is based on the theory of supporting role of stem cells through paracrine actions.[8] Stem cells are known to secrete several important growth factors, particularly EGF and IGF-I.[8,15,16] These factors have been proved to be able to effectively induce meiosis in bovine oocytes their presence could also initiate other effects such as enhancing oocyte survivability and SOD2 expression which also enhance the resiliency of oocyte toward cellular stresses.[11]

Regarding the maturation effect of CM-MSC, our findings further confirm a few reports about the efficacy of this media as oocyte culture medium. Ling et.al first suggested that CM-MSC had very high maturation effect when compared to other mediums such as alpha-MEM, DMEM, and human tubal fluid.[12] The maturation rate was reported at 91.2% compared with 63.5% in alpha-MEM, 54.7% in DMEM, and only 27.1% in HTF. Then, latest report from Akbari et.al further confirmed this finding and they also reported a significant increased in anti-apoptotic gene BCL2 and SOD

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while BAX expression was significantly decreased.[11] However, both studies did not evaluate the production of oocyte factors and this gap was filled by our study.

Despite the clear novelty of our findings, several shortcoming regarding this study are need to be considered. Our findings could not directly translated or directly used to translate the application of CM-MSC in clinical practice as further study is needed to confirm the viability of the embryo produced from oocyte that has been prepared using this medium. Using human oocytes in such experiment is not ethical and the only option is by using animal study. The actual level of BMP15 and GDF9 were also not directly evaluated in this study. The qPCR that being used in this study only provide the copy number estimation of mRNA and not actual protein. Although most of the mRNA could be translated, it is good to validate this findings by assessing the level of GDF9 and BMP15 protein directly using a method like ELISA.

CONCLUSION

According to our results, it can be concluded that incubation with CM-MSC was effective in inducing oocyte maturity with the best outcomes were observed at 24-hours incubation. However, other significant contributing factors also need to be considered such as infertile etiology, insulin resistance, and maternal age. Our model showed that the best potential candidate for the intervention to maximize the outcome is subject with insulin resistance with low risk maternal age group and does not have either PCO without syndrome or PCOS condition.

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AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article.

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CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest regarding the publication of this article.

REFERENCES

1. Bennett L, Wiweko B, Hinting A, Adnyana I, Pangestu M. Indonesian infertility patients' health seeking behaviour and patterns of access to biomedical infertility care: an interviewer administered survey conducted in three clinics. Reproductive Health. 2012;9(1). http://dx.doi.org/10.1186/1742-4755-9-24
2. Cano F, García-Velasco J, Millet A, Remohí J, Simón C, Pellicer A. Oocyte quality in polycystic ovaries revisited: Identification of a particular subgroup of women. Journal of Assisted Reproduction and Genetics. 1997;14(5):254-261. http://dx.doi.org/10.1007/BF02765826
3. Urman B, Tiras B, Yakin K. Assisted reproduction in the treatment of polycystic ovarian syndrome. Reproductive BioMedicine Online. 2004;8(4):419-430. http://dx.doi.org/10.1016/s1472-6483(10)60926-1
4. De Vos A, Van de Velde H, Joris H, Van Steirteghem A. In-vitro matured metaphase-I oocytes have a lower fertilization rate but similar embryo quality as mature metaphase-II oocytes after intracytoplasmic sperm injection. Human Reproduction. 1999;14(7):1859-1863. http://dx.doi.org/10.1093/humrep/14.7.1859
5. Lee H, Barad D, Kushnir V, Shohat-Tal A, Lazzaroni-Tealdi E, Wu Y et al. Rescue in vitro maturation (IVM) of immature oocytes in stimulated cycles in women with low functional ovarian reserve (LFOR). Endocrine. 2015;52(1):165-171. http://dx.doi.org/10.1007/s12020-015-0744-1
6. He A, Wang S, Chen T, Li J, Liu X. Biological Characteristics of MSCs. Advanced Topics in Science and Technology in China. 2009;:19-58. http://dx.doi.org/10.1007/978-3-540-88150-6_3
7. Laqif A. Kajian Terapi Media Terkondisi Sel Punca Mesensimal (MT-SPM) Selaput Amnion pada Kasus Kegagalan Ovarium Prematur (Penelitian pada Hewan Coba Tikus Sprague-Dawley) [Ph.D]. Gadjah Mada University; 2015.
8. da Silva Meirelles L, Fontes A, Covas D, Caplan A. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine & Growth Factor Reviews. 2009; 20(5-6):419-427. http://dx.doi.org/10.1016/j.cytogfr.2009.10.002
9. McNatty K, Moore L, Hudson N, Quirke L, Lawrence S, Reader K et al. The oocyte and its role in regulating ovulation rate: a new paradigm in reproductive biology. Reproduction. 2004;128(4):379-386. http://dx.doi.org/10.1530/rep.1.00280
10. Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: a systematic review of the literature. Human Reproduction Update. 2010;17(1):34-45. http://dx.doi.org/10.1093/humupd/dmq029
11. Akbari H, Eftekhar Vaghefi SH, Shahedi A, et al. Mesenchymal Stem Cell-Conditioned Medium Modulates Apoptotic and Stress-Related Gene Expression, Ameliorates Maturation and Allows for the Development of Immature Human Oocytes after Artificial Activation. Genes (Basel). 2017;8(12):371. Published 2017 Dec 8. doi: 10.3390/genes8120371
12. Ling B, Feng DQ, Zhou Y, Gao T, Wei HM, Tian ZG. Effect of conditioned medium of mesenchymal stem cells on the in vitro maturation and subsequent development of mouse oocyte. Braz J Med Biol Res. 2008 Nov;41(11):978-85.
13. Miraki S, Mokarizadeh A, Banafshi O, et al. Embryonic Stem Cell Conditioned Medium Supports In Vitro Maturation of Mouse Oocytes. Avicenna J Med Biotechnol. 2017;9(3):114–119.

14. Maldonado M, Huang T, Chen J, Zhong Y. Differentiation Potential of Human Wharton's Jelly-Derived Mesenchymal Stem Cells and Paracrine Signaling Interaction Contribute to Improve the In Vitro Maturation of Mouse Cumulus Oocyte Complexes. Stem Cells Int. 2018;2018:7609284. Published 2018 Oct 11. doi: 10.1155/2018/7609284
15. Sakaguchi M, Dominko T, Leibfried-Rutledge ML, Nagai T, First NL. A combination of EGF and IGF-I accelerates the progression of meiosis in bovine follicular oocytes in vitro and fetal calf serum neutralizes the acceleration effect. Theriogenology 2000; 54: 1327-1342.
16. Sakaguchi M, Dominko T, Yamauchi N, Leibfried-Rutledge ML, Nagai T, First NL. Possible mechanism for acceleration of meiotic progression of bovine follicular oocytes by growth factors in vitro. Reproduction 2002; 123: 135-142.