Research Article
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Comparison of two commercial *in-vitro* maturation culture systems with standard IVM media: what works best for mouse oocytes

Abstract

Background: Despite many variations made to the standard IVM protocol and to the culture conditions the rate of implantation ranges between 5% to 22% and the pregnancy rate ranges from around 8% to 40%.[1] The culture media is important as it provides the oocyte with vital metabolites such as ATP and pyruvate.[2] In order to improve the success rates with IVM, many changes have been made to the standard IVM media and studies have emerged that compare the various components of the IVM media based on maturation, developmental and fertilization rates of immature oocytes.

Aim: To determine and device an optimal *in-vitro* maturation media which is best suited to enhance IVM outcome (maturation rates of MII oocytes and subsequent embryonic development post-IVF) by culturing immature F1 mouse oocytes at the GV stage using different commercially available and homemade IVM media recipe. This study was conducted based on the hypothesis that diversity in the various commercially available and in-house IVM media can have a significant potential effect of the maturation rate of oocytes.

Methodology: A sum total of (273) GV oocytes were collected from F1 mice, out of which 261 GV oocytes were available for *in-vitro* maturation which were randomly allocated to and cultured in IVM media (LAG/NON-LAG) (n=93/85) and a control group (n=95) cultured in a standard or in-house IVM recipe (Appendix A). Supplemented with FCS or fetal Calf Serum (0.5ml), PMSG (3µL), HCG (10µL) penicillin and streptomycin (1ml/100ml IVM media stock), EGF (2µL), Na-pyruvate (2µL), PMSG (10µg/ml), HCG (5µg/ml). We then proceeded to assess the maturation rates of the immature oocytes up to the M-II stage at 17 hours post culture in maturation media. Furthermore, the developmental competence of the *in-vitro* matured M-II stage oocytes were also assessed by performing IVF of these oocytes and assessing outcome measures such as fertilization potential and subsequent culture until day 5 with daily observation of cleavage rate and development up to blastocyst stage. All procedures were performed according to commercially available protocols as per the manufacturer's instructions.

Results: The following results were obtained in terms of maturation rate (34/95:35.79% & 31/93: 33.33% and 24/85: 28.2% respectively) and the fertilization rate (12/95: 35.29% & 9/93: 29.03% & 4/85: 16.7%) each of which met the expected values Chi-squared=1.99; with a degree of freedom=2. This result was found to be statistically not significant ($p<0.6$) in each of the groups respectively.

Discussion/interpretation: Variation in the IVM culture systems does not seem to affect the maturation rate and subsequent fertilization outcomes.

Limitations: A small sample size and a limited number of replicates performed for each of the available IVM culture systems.

Conclusion: Conclude that the modified Medicult IVM system may not be the best choice of media for maturing F1 mouse oocytes along with cumulus cells *in-vitro*.

Keywords: *in-vitro* maturation, oocytes, fetal calf serum, blastocyst, medicult

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Abbreviations: IVM, *in-vitro* maturation; COH, controlled ovarian hyper Stimulation; RHI, recombinant human insulin; LPA, lysophosphatidic acid; EGF, epidermal growth factors; BCM, blastocyst culture media; GV, germinal vesicle; eCG, equine chorionic gonadotropin; PMSG, pregnant mare serum gonadotropin; PB1, first polar body; FSH, follicle stimulation hormone; ITS, insulin along with transferrin and selenium

Introduction

Pincus & Enzmann[3] were the first researchers to carry out *in-vitro* maturation (IVM) of the oocyte by using the animal model. Data gathered from many centres have suggested that IVM is a safe and an effective approach to ART.[4–7] Given the fact that considerable progress with IVM has been made in the past decade there still remains an insufficiency of data pertaining to the fertilization rates

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of IVM oocytes, ongoing rate of pregnancy and live birth rate.[8] The transfer of IVM embryos has produced a viable offspring as stated earlier by Son BY.[9]

At the time of Controlled Ovarian Hyper stimulation (COH) around 5% to 15% of the oocytes retrieved fail to completely reach nuclear maturity[10] therefore *in-vitro* maturation was implemented in these scenarios to try and mature oocytes *in-vitro* to maximize oocyte yield especially in the case of poor responders such as E2-resistant patients.[11] Further investigation on improving the technique of IVM is crucial as it offers a wide range of benefits to patients such prevention of OHSS and allowing the transfer of embryos during a natural or unstimulated cycle.[12]

The role of culture media is to promote oocyte growth and survival *in-vitro* and also to maintain nuclear and cytoplasmic maturation of the oocyte. Therefore Vitamins have recently been included as part of the Medicult IVM system which comprises of a pre-IVM or LAG phase (Appendix B) pH and osmolality buffers, ions etc. to fulfil these requirements. Additionally the Origio Medicult IVM media system also contains human serum supplements and recombinant Human Insulin (RHI).[13] Certain extracellular signalling molecules such as lysophosphatidic acid (LPA) in an amount of 30µm to TCM-199 medium in case of the mouse oocyte has been associated with an increased maturation rate (94.3%) and blastocyst formation rate (79.1%) as reported by Jo JW et al.[14]

An ideal IVM media should comprise of epidermal growth factors (EGF) when directly culturing COCs or cumulus oocyte complexes for *in-vitro* maturation.[15] Oocyte maturation is induced and enhanced by the further addition of growth factors (EGF), serum (FBS, HSA), steroids and growth hormones for example, EGF is a naturally occurring growth factor present in the ovary and is linked to an enhanced maturation rate in case of the human GV oocytes.[16] In order to be able to fulfil specific requirements of the developing oocyte *in-vitro*, specific *in-vitro* maturation media such as the SAGE IVM Cooper Surgical kit, ART-1600) and ORIGIO Medicult IVM system 8221 are supplemented with the epidermal growth factor (EGF), hCG, FSH/LH and growth hormones.[17] EGF is high gonadotropin dose dependence.[18] EGF is usually added at a concentration of 0.0610mg/L along with FSH/LH (75IU each). A previous study on the Rhesus monkey Callithrix Jacchus oocyte, by Tkachenko et al.[19] who reported that EGF in combination with a low dose of gonadotropin supplementation was found to have detrimental effect of the oocyte maturation rate. Although most of the media companies are supplemented with serum, it is still recommended to include EGF as a part of the media composition just before the time of use.[20] Given that EGF plays a vital role as a media component, it was used as a supplement in our standard IVM recipe as well as a part of the modified Origio Medicult system in the present study.

Standard IVM media (origio A/S, Jyllunge, Denmark) has shown to provide good results previously[9,21] for instance, TCM-199 and the blastocyst culture media (BCM) which are both supplemented with pyruvate and have shown to yield equally good results.[14] In contrast to these findings, another study reported that pyruvate was not found to sufficiently improve oocyte cytoplasmic maturation, thus recently sodium pyruvate has been added to maturation media in case of the bovine oocyte model.[22] Therefore, in our study we decided to include Na-pyruvate at a concentration of 0.002g/ml which was postulated to be suitable in case of the immature mouse oocyte model.

The supplementation of *in-vitro* maturation media with water soluble vitamin such as inositol was found to be associated with increased blastocyst development in the ICR mouse oocyte model according to previous literature presented by Chiu TT et al.[23] Accordingly,[24] found that by supplementing IVM media with vitamins such as the minimum essential medium or MEM at a low concentration (0.4%) improved embryo developmental potential in case of the porcine oocyte model. Based on these finds we chose to use 1.02g/100mL of MEM as a base ingredient for our standard in-house IVM recipe.

Previous findings from studies which have retrospectively compared two IVM media have reported that it is vital to supplement IVM media with a combination of steroid and gonadotropin according to Funahashi H[25] who have indicated that by using a combination of hCG & eCG (equine chorionic gonadotropin) supplementation, a significantly high rate (78.56%) of cytoplasmic maturation was achieved in case of the porcine oocyte when cultured for a period of 21hours.[26] For a longer duration of exposure of oocytes in culture for up to 42hours, an additional combination of hCG and PMSG along with follicle stimulating hormone could enhance maturation rates during extended culture time.[26] On the basis of the above mentioned findings[27,28] formulated a new IVM recipe which was proved to provide the porcine *in-vitro* maturing oocytes with a stable environment (Table 1). This IVM media comprised of the following components.

Table 1 Modifications made to IVM media to suit the conditions of porcine oocytes[27]

Ingredients	Quantity/Volume Added
TCM 199- Suppl. With	Polyvinyl Alcohol (0/1%); Cystein (0.57mmol/l); Glucose (3.05mmol/l); Pyruvic acid (0.91mmol/l); Na-salt (mM 199)
Epidermal Growth Factor (EGF)	10ng/ml
PMSG	10IU/mL
hCG	10IU/mL
FSH	2.5IU/mL
(Insulin-transferrin-selenium)ITS	1%

Thus far, various companies have introduced different IVM media systems with a combination of ingredients. At present, further studies need to be conducted to test the efficacy and reliability of these products. Thus, the current study aimed to evaluate the maturational rate by assessing the efficacy of three IVM media which were either commercially available or produced in-house to be suited for our laboratory conditions in case of the immature mouse oocyte at the germinal vesicle (GV) stage.

According to the manufactures of (ORIGIO Medicult IVM system: OSE: MEC), this system contains cholesterol inhibitors (statins) which aid in stimulating meiosis in case of mine. The system also possesses a prostacyclin molecule which has been shown to have a favourable effect on pre-implantation human oocytes and embryos (unpublished data). Thirdly, the system includes (GM-CSF), a growth factor linked to increased blastocyst hatching rates, improved pregnancy outcome.

It is necessary that these unpublished data be validated. For the same reason, animal models have been used on a large scale and their findings have been further extrapolated in case of human oocytes. Therefore, this study aims to assess the maturation rate, subsequent developmental rate post IVM and IVF of embryos derived from *in-vitro* cultured & matured M-II stage mouse oocytes. We hypothesized

that the newly designed Medicult IVM system (ORIGIO) would be able to potentially enhance the maturation rate and subsequent embryo development post fertilization , provided that its superiority has been indicated according to previous literature in case of other animal models as well as in case of human in comparison with the standard IVM media. The rationale behind the study was that additional supplementation of the Medicult IVM system with a variety of combinations of constituents would have a positive impact on the *in-vitro* maturation process in case of the *in-vitro* matured F1 mouse oocyte.

We cultured 261 immature mouse oocytes at the GV stage which were randomly allocated to two test group in NON-LAG and LAG IVM media (Medicult IVM System) in comparison to a control group matured in-house IVM media. We then proceeded to assess the maturation rates of the immature oocytes up to the M-II stage at 17hours post culture in maturation media (Table 2). Furthermore, the developmental competence of the *in-vitro* matured (IVM) M-II stage oocytes were also assessed by performing IVF of these oocytes and assessing outcome measures such as fertilization potential and subsequent culture until day 5 with daily observation of cleavage rate at day 3 up until the development to a day-5 or at the blastocyst stage. All procedures were performed according to commercially available protocols as per the manufacturer's instructions.

Table 2 Demonstrates the maturation rate (35.79%) and fertilization rate (35.20%) along with subsequent development up to the blastocyst stage at day 5 post culture in Group A, home-made media

Group A, Homemade IVM Media			
Date	**No. of GV oocytes**	**MII oocytes**	**No. of fertilised oocytes**
7-Aug	67	21	8
17-Aug	20	8	2
19-Aug	8	5	2
Total	95	34	12
	Rate	**Lower Limit**	**Upper Limit**
% MII	35.79	35.69	35.89
% Fert	35.29	35.2	35.39
% Arrested	64.03		

The present study may be considered as a stepping stone towards the comparison of various commercially available IVM media and develop a better understanding towards optimizing the IVM media which may potentially be designed in future to be suited for culturing immature mouse oocytes. Wider implications of this study could be to enhance maturational rates and quality of immature GV and MI stage oocytes to be cryopreserved at the immature stage. Further studies could be directed toward the study of blastocyst development post vitrification and warming of *in-vitro* matured mouse oocytes.

Material and methods

Ethics

"All animals that were used as a part of this study were approved from Monash Medical Centre Animal Ethics Committee (AEC approval number MMCA2011/84)".

Mice

F1 strain of female mice was injected intra-peritoneally with 1.5mL of Pregnant Mare Serum Gonadotropin (PMSG) by folligon, Bendigo. Female mice were then sacrificed 48hours later by the method of cervical dislocation.

Chemicals & IVM media composition

Two types of commercially available *in-vitro* maturation media by Origio Medicult IVM system comprising of (IVM media in the presence / absence of a LAG/NON-LAG media) (Appendix B) was modified with additional supplementation of FCS or fetal Calf Serum (0.5ml), PMSG (3µL), HCG (10µL) and was compared with a standard IVM media recipe which is designed to support the growth of immature mouse oocytes *in-vitro*. The standard IVM media (Appendix A) was supplemented with combination of antibiotics; penicillin and streptomycin (1ml/100ml IVM media stock), EGF (2µL), Na-pyruvate (2µL), PMSG (10µg/ml), HCG (5µg/ml).

Pilot study

A single trial was conducted before conducting this study which aimed at enhancing the user's ability to carry forward the necessary procedures which were to be conducted as a part of this project.

Experimental design

GV oocyte collection

A total number of (273) sibling oocytes at the germinal vesicle or GV stage were collected in a modified Simplex potassium based (KSOM) handling media (Appendix C) as previously described by Roesner S et al.[8] was utilized for handling during collection and dissection of ovaries and retrieval of GV oocytes from ovarian follicles under an (Olympus SZ51) dissecting microscope, while maintaining aseptic and sterile conditions. Culture dish containing 30 micro-litres of *in-vitro* maturation (IVM) media with an overlay of mineral oil was prepared and pre-equilibrated 24hours prior to collection in a Hera incubator at 37° Celsius with 5% each of carbon-di-oxide and oxygen gas combination.

IVM & oocyte allocation

Freshly collected GV stage sibling oocytes were transferred in Mouse oocytes containing a germinal vesicle and an intact nucleus and homogeneous cytoplasm were selected and randomly allocated to 3 different type of IVM media comprising of the following three groups: Group A: standard/ home-made IVM media recipe; Group B: Lag ORIGIO Medicult System and Group C: Non-Lag ORIGIO IVM Medicult System and were rinsed in IVM culture media and ≤10 GV oocytes were placed in individual drops and were incubated within a Hera incubator at 37° Celsius with 5% each of carbon-di-oxide and oxygen gas combination.

Assessment of oocyte maturation

Oocyte maturation status was recorded 16-18hours post culture in IVM media. The oocytes that exhibited the extrusion of the first polar body (PB1) were identified, counted in terms of number of oocytes matured/ total number of GV oocytes present in a single drop.

in-vitro fertilization (IVF)

Epidiymal tissue was collected with an adult F1 male mouse post cervical dislocation. Each dissected epididymal tissue was placed in two separate tubes of 1mL of KSOM MT6 media (Appendix B) pre-equilibrated in a Hera incubator with a loose lid for swim up for 30minutes. M-II oocytes were transferred to a 2 X 50µL of MT6 media drop in a culture plate filled with 6mL of mineral oil which were pre-equilibrated overnight and were subsequently inseminated with a required volume of prepared sperm sample post calculation of estimated motility (%) and Number of sperm/mL on a Makler Chamber by counting 10 rows and columns respectively. Using the % motility and sperm count/mL measured, the sperm volume for insemination was determined by using the following formula: Concentration of motile sperm= % motility X concentration of sperm/mL. The calculated sperm volume for insemination in (µl) was aliquoted from the MT6 + sperm mixture and placed into each 50µl drop of MT6 containing oocytes. The time of insemination was noted and each of the MT-6 droplets was checked 7-8 hours post insemination for signs of 2 pronuclei (2PN) and 2 polar bodies (2PB). The two-cell embryos were then transferred to pre-equilibrated culture plates containing 30µL droplets of ISM1™ culture media (ORIGIO) (Appendix D) 48 hours post culture, the cleavage stage embryos were transferred to changeover blastocyst culture media (Appendix E, Blast Assist® culture media, ORIGIO).

Statistical analysis

Statistical analysis was performed by using Microsoft Excel. Chi-squared test was used to analyse the variability of oocyte maturation rates (%) among the three treatment groups. A p value of less than 0.5 was considered as statistically significant.

Results

A sum total of 261 immature mouse oocytes at the GV stage were available to be randomly allocated into the following three groups (Group A: 95, Group B:93 & Group C:85) were matured up to the M-II stage and analysed 17hours post culture. The following results were obtained in terms of maturation rate (34/95: 35.79% & 31/93: 33.33% and 24/85: 28.2% respectively) and the fertilization rate (12/95: 35.29% & 9/93: 29.03% & 4/85: 16.7%) (Table 3). The maturation rate (%) of (Group A: 34% vs. 30.97%; Group B: 31% vs. 30.32% & Group C: 24 vs.27.71) & arrest rate (%) of (Group A: 61% vs. 64.03%; Group B: 62% vs. 62.68% & 61% vs. 57.29%) each of which met the expected values CHI-squared=1.99; with a degree of freedom=2. This result was found to be statistically not significant ($p<0.6$) in each of the group respectively (Table 4). Thus, the results obtained among Group A, B and C when comparing the total rate (%) of arrest at the different stages of embryonic development demonstrate that the highest arrest rate was observed in Group B (Lag medicult Media at 62% vs. group A=61% and group C=62%). However, this difference again, was found to be statistically not significant ($p<0.6$) CHI-squared value=1.99; with a degree of freedom=2.

Table 3 Demonstrates the maturation rate (%) and fertilization rate (%) along with subsequent development up to the blastocyst stage at day 5 post culture in Group B, Medicult IVM media system with a LAG/PRE-IVM phase culture

Group B, lag (origio)			
Date	**No. of GV oocytes**	**MII oocytes**	**No. of fertilised oocytes**
17-Aug	11	5	2
19-Aug	20	9	2
21-Aug	25	5	2
26-Aug	37	12	3
Total	93	31	9
	Rate	**Lower limit**	**Upper limit**
% MII	33.33	33.24	33.43
% Fert	29.03	28.94	29.12
% Arrested	62.68		

Table 4 As demonstrated in the following tables, the outcomes in terms of maturation rate (%) and the arrest rate (%) were found to match with the expected values with a CHI-squared value=1.9; degree of freedom=2 in each of the three groups compared with no significant difference respectively ($p<0.6$)

Group C, Non-lag			
Date	**No. of GV oocytes**	**MII oocytes**	**No. of fertilised oocytes**
17-Aug	13	3	0
19-Aug	17	5	2
21-Aug	18	4	1
25-Aug	37	12	1
Total	85	24	4

Table continued...

Group C, Non-lag			
Date	**No. of GV oocytes**	**MII oocytes**	**No. of fertilised oocytes**
	Rate	**Lower limit**	**Upper limit**
% MII	28.2	28.1	28.3
%Fert.	16.7	16.6	16.7
% Arrested	57.29		

Table 5 Demonstrates the maturation rate (%) and fertilization rate (%) along with subsequent development up to the blastocyst stage at day 5 post culture in Group B: Medicult IVM media system without pre-IVM or (NON-LAG) phase culture

Outcome(%)	Group A, homemade	Group B, lag	Group C non lag	Total
MII stage	34	31	24	89
Arrested	61	62	61	184
Total	95	93	85	273

Expected			
	Homemade	**Lag**	**Non lag**
MII stage	30.97	30.32	27.71
Arrested	64.03	62.68	57.29

Interpretation

Overall, regarding the maturation rate, it was found that the (Group A) in-house IVM media recipe (Appendix A) was found to be better suited with a higher maturation rate (35% vs. 33.3% and 28.2% respectively) and a higher fertilization rate (35.29% vs. 29.03% and 16.7%) in comparison with Medicult system with a LAG phase + IVM phase (Group B) (Appendix F) and NON-LAG Medicult IVM system (Group C). However, these results were not observed to be statistically significant ($p<0.006$). Secondly, the low fertilization rate 16.7% as shown in (Table 5) using the non-lag medicult media system is a challenge to explain as it could be a result of user variability or the IVM procedure itself, along with media constituents and their relative effects on the mouse GV oocytes.

Discussion

Edwards in 1965 described the composition of the earliest IVM culture systems used in case of the mouse model which was the Waymouth's Medium, MEDIA-199 and Hank's saline supplemented media consisting of human and/or fetal calf serum, antibiotics, a bicarbonate buffer maintained at a pH value of 7.2. Mouse oocytes were treated with Difco Medium-199 which was supplemented with 15% serum.

The two most commonly used IVM media in most clinics across the world are TCM-199 and Ham's F-10 media as reported by Mikkelsen AL,[4] Cha KY et al.[6] Thereafter, many retrospective studies have been carried out to assess the efficacy of the various commercially available IVM media on the animal as well as human oocytes. Recently[29] found no significant difference in the total maturation rate of human oocytes on treatment with TCM-199 and Medicult IVM system (Figure 1). In accordance with these studies, similar results were obtained among the standard media and the modified Medicult IVM system (Origio) (Appendix F) as we found no significant difference in the maturation rates and fertilization rates among all the study groups in case of the F1 mouse oocytes.

In the present study a high rate of meiotic arrest at the two-cell stage as well as at cleavage stage was observed in all the groups (data not shown) which may have been linked to previous findings by Edwards in 1965 suggesting that a drastic reduction in maturation rate could have occurred as a consequence of meiotic arrest at anaphase-I which was linked with an increase in pH, antibiotic concentration of the media to around 1,000IU/ml (Appendix G). Therefore, this indicates that various combinations *in-vitro* maturation media constituents need to be researched up on to further develop and optimize the IVM culture systems in case of the mouse model.

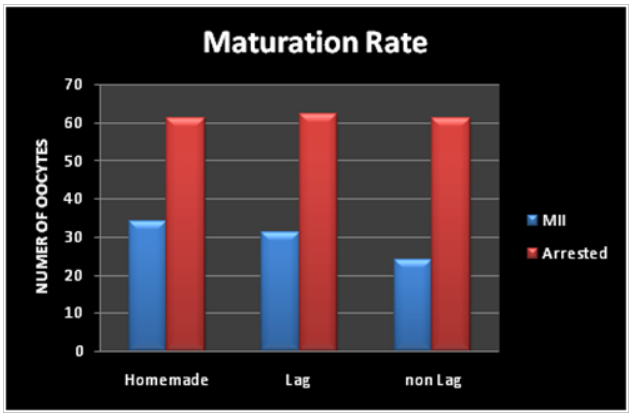

Figure 1 Maturation rate in group A (Homemade), group B (Lag) and group C (Non-group C) respectively.

The SPOM system or the stimulated physiological oocyte maturation system described by Albuz FK et al.[30] in murine and bovine model is based on a theory that the precocious resumption of meiosis is prevented with introduction of a pre-IVM phase which includes a non-specific inhibitor (FSK+IBMX) that increases cAMP levels but only within the CCs and allows sufficient time for the oocyte to gain complete developmental competence. This Rise in cAMP at the CC during pre-IVM is loaded in to the intraoocyte components via prolonged gap junction communication during the extended IVM phase (Figure 2). Induced Intraoocyte rise in the cAMP levels is regulated with cilostamide which blocks the action of cAMP modulators thereby inhibiting oocyte maturation. Additionally, the inductive effect of FSH overrides the inhibiting effect of cilostamide.

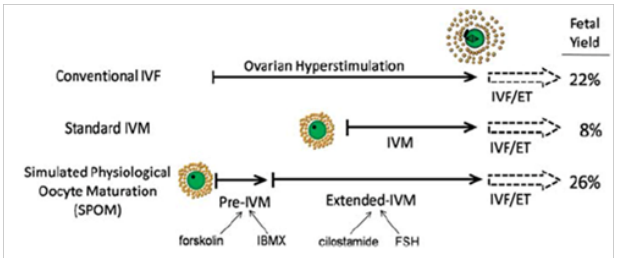

Figure 2 SPOM is associated with,A doubled rate of fetal yield of 26% vs. 22% comparable to IVF results (P>0.05).

Thus, the rationale behind using a LAG phase or a pre-IVM phase could be potentially to, delay or prolong the process of spontaneous oocyte maturation from occurring during IVM as a consequence of the process of mechanical aspiration and removal of the surrounding cumulus cells. As previously reported by Cheng Y,[31] both mouse strain and culture environment have a significant difference in meiotic defects and spontaneous activation. Changes in culture media were associated with strain specific responses to the culture environment (Appendix H). Supplementation of the IVM media with follicle stimulation hormone (FSH) in the presence of cumulus cells stopped the process of spontaneous meiotic division from occurring in case of the C57BL/6 strain but not in the F1 strain of mice. We therefore conclude that the modified Medicult IVM system used as a part of this study may not be the best choice of media for maturing F1 mouse oocytes along with cumulus cells *in-vitro*.

For the purpose of improving the technique of IVM, a considerable emphasis has been laid on the optimization of culture media systems to enhance the *in-vitro* maturation rate. For this purpose, numerous manufacturing companies such as (SAGE IVF, Medicult & Cook) have formulated a variety of new culture media systems comprising of varying concentrations as well as contents to formulate a new IVM recipe which would be best suited for the human oocyte development *in-vitro*. For example, the Medicult IVM system comprises of a pre-IVM media or the LAG-phase media which is additionally supplemented with recombinant human insulin. According to Lee MS et al.[32] insulin may have a potential positive effect in enhancing the cleavage rate and subsequent developmental outcomes in case of the porcine oocytes cultured *in-vitro*. Furthermore, a combination of insulin along with transferrin and selenium (ITS) which was used as a serum supplement in this study, is suitable in case of the immature mouse oocytes as previously stated by De La Fuente R,[33] Cobo AC,[34] De Moraes-Ruehsen M,[35] Fair T,[36] Thomas RE,[37] Vaccari S et al.[38] as a combination of these three molecules aid in enhancing glucose uptake in addition to inducing the detoxification of ROS.

Conclusion

In conclusion, this study reported that variation in the IVM culture systems do not seem to affect the maturation rate and subsequent fertilization outcomes. However, this study was limited by constraints such as a small sample size and a limited number of replicates performed for each of the IVM culture systems. Therefore, further research should be carried out which includes a larger sample size in order to assess fertilization outcomes in order to be able to draw a comparative analysis with previously established gold standard literature in case of the mouse oocyte. The factors affecting the process of oocyte maturation and subsequent fertilization rates followed by embryonic development and pregnancy outcomes are: source of oocyte, intra-species specific variation among oocytes and culture conditions.[9] Thus, given the non-significant results as above mentioned, it would be worthwhile to conduct further studies to determine whether the recipe used as a part of this study, would be able to yield positive result when extrapolated in case of other mammalian oocytes. The next step forward would be to enhance the maturation outcome obtained from GV stage oocytes in terms of quality and quantity, to be able to cryopreserve these *in-vitro* matured oocytes.

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Conflict of interest

Author declares that there is no conflict of interest.

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