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Evaluation of fertilization medium quality in in vitro maturation of human germinal vesicle oocytes gained during intra cytoplasmic sperm injection cycles

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Abstract---Background: in vitro maturation of human germinal oocytes is a promising technique in assisted reproductive technologies, different medium has been introducing in last two decays for this purpose but they are very expensive and gained by order with different qualification Objective: the aim of study to evaluate the efficacy of fertilization medium used in in vitro maturation of germinal vesicles gained during ICSI cycles. Patients and methods: 42 oocytes in germinal vesicle stage involved in in vitro maturation (IVM) program with fertilization medium, they represent 22 cases (couples) that underwent IVF-ICSI operation, gained from them, (9) of them excluded because they are morphologically abnormal, the rest (33) GV introduced in program, they incubated for 24 hours and reassessed again to evaluate their maturation, the study done in the High institute for infertility diagnosis and assisted reproductive technologies, Al- Nahrain University from the period December 2018 to February 2020 . Results: out of (33) Gv incubation for 24 hours (6) of them remain in GV stage, (8) of them pass to M1 maturation stage, and another (8) passes to M2 stage of maturation, the rest 11 they were atretic. (6) oocytes in GV stage represent (18.18 %) of total number and (8) oocytes in M1 stage represent (24.24%), (8) oocytes in M2 stage represent (24.24 %) while (11) was atretic represent (33.33%). The residual (6) oocytes in GV and (8) in M1 stages incubated for another 24 hours the results were as the following: For the GV: (2) of them remain in GV stage and represent

(33,33 %) of incubated GV, and (1) of them transform to M2 represent (16.67 %). and (3) of them become atretic stage represent (50 %) of them. For M1 oocytes: (1) of them remain in M1 stage represent 12,50 %, (2) of them converted to M2 stage represent 25 % of them and the rest (5) become atretic represent 62,5 %. the result of the overall incubated period (24-48) hours was as the following:(2) GV oocytes remain in GV stage of maturation represent (6.06 %) of total oocytes incubated, (1) oocytes was in M1 stage of maturation represent (3.03%) and (11) oocytes pass to M2 stage of maturation represent (33.33%) the rest 19 oocytes represent (57.58%) has been atretic conclusion: Fertilization medium may be useful in in vitro maturation process for human germinal vesicle and may be as alternative for ordinary IVM medium in ICSI cycles.

Keywords---Reproductive technologies, Fertilization medium, IVM.

Introduction

In vitro maturation (IVM) of human oocytes is a more recent procedure than traditional IVF of oocytes, having been first used successfully in 1991 (Cha et al., 1991), whereas IVF was first applied successfully to humans in 1978 (Steptoe and Edwards, 1978). However, IVM only became generally used in the 2000s in human oocytes (Michael H.eatal.,2016).

Immature oocyte retrieval followed by in vitro maturation (IVM) opens a new distance for modern assisted reproductive technologies (ART). Recent studies in IVM make it a possible alternative to in vitro fertilization. IVM refers to the maturation of immature oocytes in culture after their recovery from small antral follicles at the stage earlier to selection and dominance (Yu-Hung L. and Loung H. 2006). Occasionally, however, retrieval of oocytes at the germinal vesicle (GV) or metaphase I (MI) stage represents a significantly large portion of the oocytes retrieved during IVF –ICSI cycle (David E. Reichman.2010). After ovarian stimulation (in ART procedures) with gonadotropin and HCG (human chorionic gonadotropin) introducing for final maturation, about 15% of oocytes are found in germinal vesicle (GV) or metaphase I (MI) at the time of oocyte retrieval (Panel RC. and Chian S.2002). Oocytes that are immature at the time of the cumulus cell removal can acquire, in vitro, the nuclear and cytoplasmic maturity that yields the ability to be fertilized and develop (David B.eatal.,2015).

The in vitro maturation of oocytes is mainly affected by culture conditions. the common media used for the IVM of immature human oocytes include (tissue cell media) TCM-199 medium, Ham's F10 medium, and Chang's medium. In addition, serum, gonadotropin ,follicle-stimulating hormone and luteinizing hormone , growth factors, and steroids can be added in a basal medium to produce a complex medium. At present, commercial IVM media have been widely used (Mark Hill 2016).

In vitro maturation medium can be broadly divided into simple and complex. Simple media are usually bicarbonate-buffered systems containing physiological

saline with pyruvate, lactate and glucose, and they differ in their ion concentration and in the concentrations of the energy sources. Complex media contains in addition to the basic components of simple media, amino acids, vitamins and purines, Steroids, Gonadotropins, Growth factors, cytokines and follicular fluid (Pallop Pongsuthirak¹ and Teraporn Vutyavanich² 2014, Pallop P., Sorramon S., and Teraporn V.2015).

Subject, Materials and Methods

42 oocytes in germinal vesicle stage involved in in vitro maturation (IVM) program with fertilization medium, they represent 22 cases (couples) that underwent IVF-ICSI operation, gained from them, (9) of them excluded because they are morphologically abnormal, the rest (33) GV introduced in program, they incubated for 24 hours and reassessed again to evaluate their maturation, the study done in the High institute for infertility diagnosis and assisted reproductive technologies, Al- Nahrain University from the period December 2018 to February 2020 . The oocytes collected from the female's patients by oocyte pickup under general anesthesia or spinal anesthesia, after pick up the oocyte with their cumulous cells transfer to special medium (preparation medium) and put for at least 2-3 hours in 5% CO₂ incubator at 37 c. Normal GV Collected and transfer to the media (maturation medium). morphologically abnormal oocytes (GV) where excluded (27) GV were abnormal) and rest normal incubated for 24 hours and re-examine again to assess their maturity.

Results

1. GV incubation for 24 hours

42 oocytes in germinal vesicle stage involved in in vitro maturation (IVM) program with fertilization medium, they represent 22 cases (couples) that underwent IVF-ICSI operation, gained from them, (9) of them excluded because they are morphologically abnormal, the rest (33) GV introduced in program, they incubated for 24 hours and reassessed again to evaluate their maturation ,they are matured in different manor as the following

(6) of them remain in GV stage, (8) of them in M1 maturation stage, and another

(8) passes to M2 stage of maturation, the rest 11 they were atretic. As showed in

Figure (1):

(6) oocytes in GV stage represent (18.18 %) of total number and (8) oocytes in M1 stage represent (24.24%), (8) oocytes in M2 stage represent (24.24 %) while (11) was atretic represent (33. 33%).as showed in figure (1)

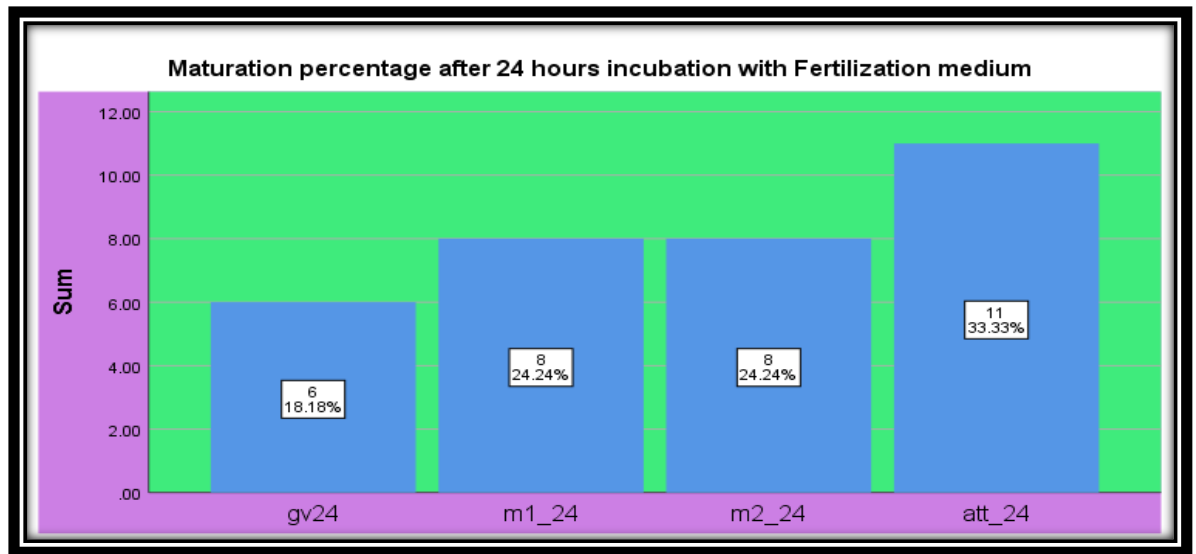


Figure (1): showed the percentage fate of GV after 24 hours' incubation with fertilization medium.

residual GV and M1 incubated for another 24hours

(6) oocytes in GV and (8) in M1 stages incubated for another 24 hours the results were as the following:

For the GV: (2) of them remain in GV stage and represent (33,33 %) of incubated GV, and (1) of them transform to M2 represent (16.67 %). and (3) of them become atretic stage represent (50 %) of them as showed in figure (2)(3)

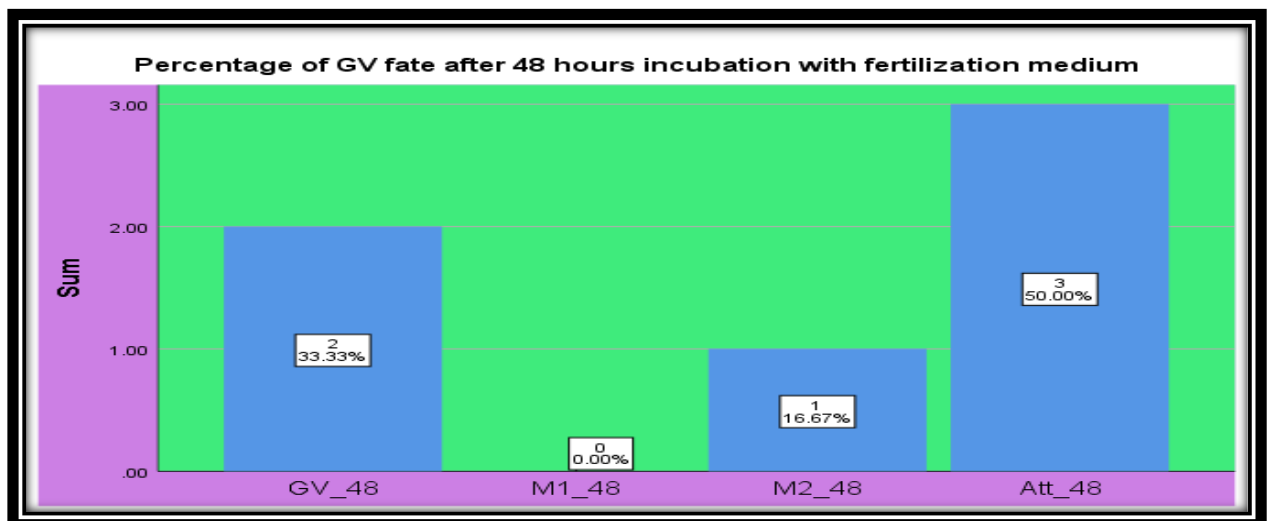


Figure (2): showed the fate and percentage of GV after 48 hours' incubation with fertilization medium.

For M1 oocytes: (1) of them remain in M1 stage represent 12,50 %, (2) of them converted to M2 stage represent 25 % of them and the rest (5) become atretic represent 62,5 %. As showed in figure (3)

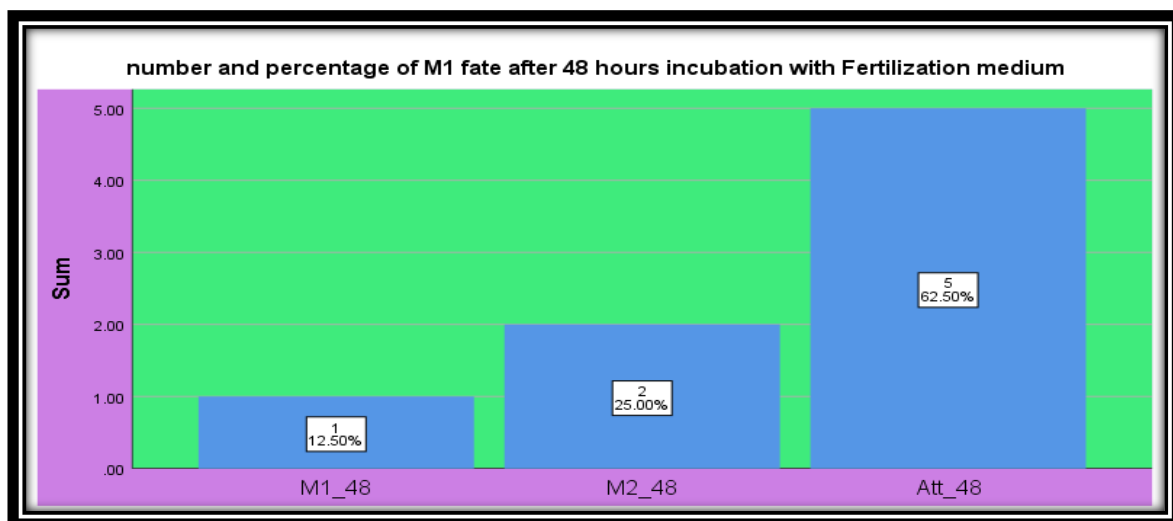


Figure (3): showed the number and the percentage of fate of M1 after 48 hours incaution with Fertilization medium.

2. Total incubated period (24-48) hours

the result of the overall incubated period (24-48) hours results was as the following: (2) GV oocytes remain in GV stage of maturation represent (6.06 %) of total oocytes incubated, (1) oocytes was in M1 stage of maturation represent (3.03%) and (11) oocytes pass to M2 stage of maturation represent (33.33%) the rest 19 oocytes represent (57.58%) has been atretic. as showed in figure (4)

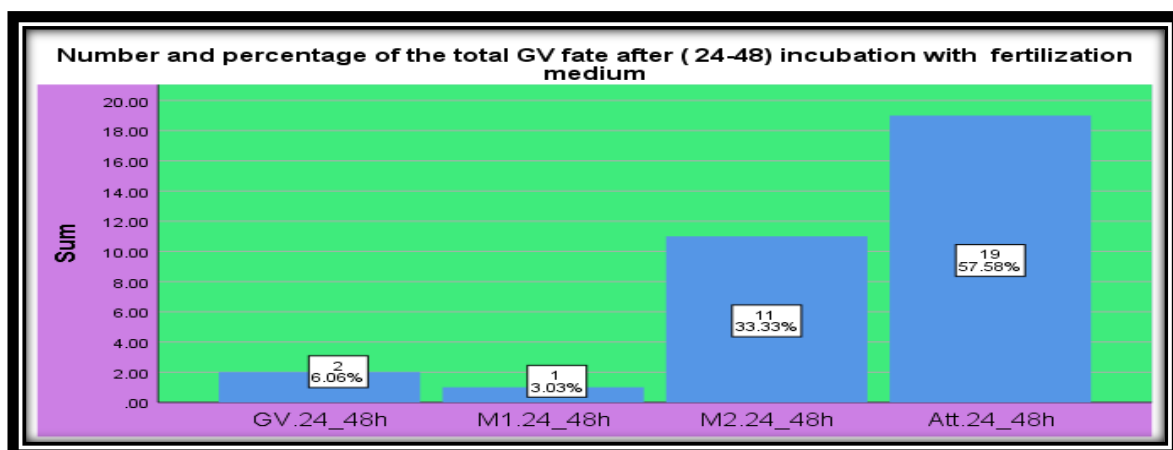


Figure (4): showed the number and percentage of the total GV fate after 24-48 hours' incubation with Fertilization medium

3. Comparison between different maturation stage and times

Results of comparison between different maturation stages and time of incubation for Fertilization medium.as showed in fig (5)

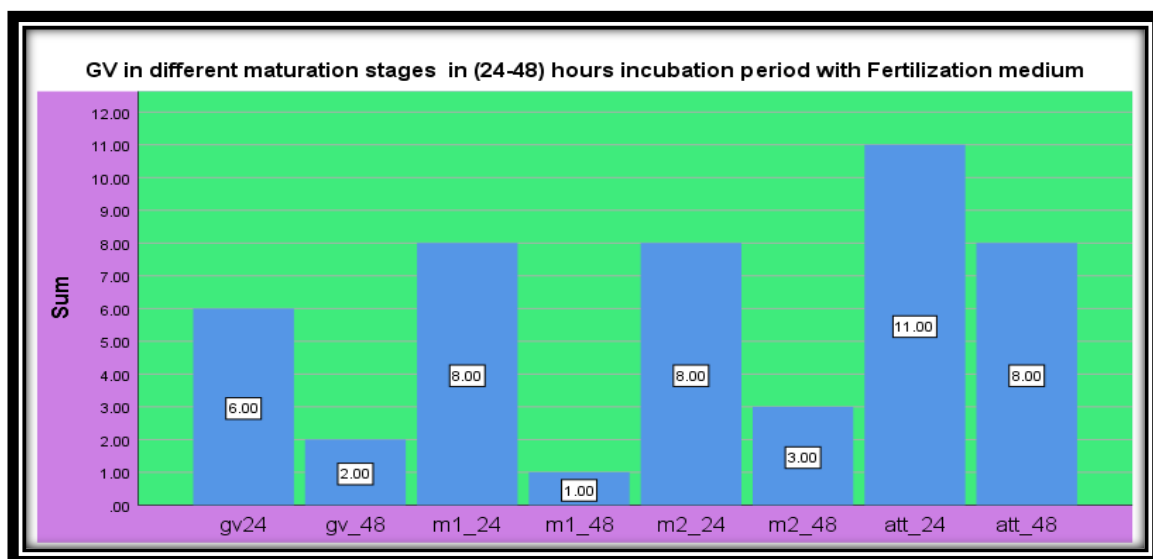


Figure (5): showed the different maturation stages of GV incubated for (24-48) hours incubation period with fertilization medium.

Paired Test	Mean	S.D	S.E. Mean	P .value	Sig.(P< 0.05)
GV/24h- GV/48h	.21053	.41885	.09609	.042	Sig
M1/24h- M1/24h	.36842	.49559	.11370	.005	Sig
M2/24h- M2/48h	.26316	.73349	.16827	.135	Not sig
Att/24h- Att/48h	.15789	.60214	.13814	.268	Not sig

Table (1): showed the descriptive statistic and results of paired t –test between 24hours and 48 hours incubation period for different maturation stages with Fertilization medium.

There is a significant difference between residual GV that remain from incubation for 24 hours and GV incubated for 48hours, (6) oocytes in GV stage represent (33.33 %) % for 24hours incubation in comparison with (2) of them remain in GV stage at 48 hours' incubation and represent 18.18 %, (p value < 0.05) for t- test.. And there is a significant difference in results of M1 stage of oocytes between 24 hours incubated period and 48hours incubated period (p value < 0.05) for t- test. significant results obtain in 24 hours' incubation (8) oocytes in M1 stage represent (24.24%) while in 48hours incubated period (1) oocytes converted to M1 stage of maturation represent (12.5%)

No statically significant difference in results of M2 stage oocytes between 24 hours incubated period and 48hours incubated period (p value > 0.05) for t- test. No statically significant difference in results of atretic oocytes between 24 hours incubated period and 48hours incubated period (p value > 0.05) for t- test

Discussion

The results may be due to inadequate number of oocytes (GV) that introduced in this section of our study, in addition to that the fertilization medium is not specialized for IVM purpose. Fertilization medium principally used to support embryo from fertilization till morula stage of embryonic development.

(Blanco MR.et al., 2011, Ri-Cheng Chian, 2018) suggested that in vitro maturation of Oocyte in Stage is intensely affected by culture environments .

Therefore, the culture medium used for IVM process should be designed precisely for oocytes development. GV maturation including cytoplasmic and nuclear growth, therefore that IVM medium consider as a complex medium. Thus there is no difference in maturation and fertilization rate of oocyte that matured in different media whom specially designed.

(Swain JE. et al., 2016, Swain JE. 2015, Swain JE and Smith GD. 2011). Founded that, provisional requirements of IVM system, program of culture stages and devices, all of them, play an important role in manipulation of undeveloped oocytes, and subsequently embryonic progression. Which agree with our study

Conclusion

Fertilization medium is less Qualified than specialized IVM medium and need more study in future to used them in Ivm purposes

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