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

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***Abstract*---**Background : Different medium has been introducing in last two decays for IVM of human oocytes germinal vesicles but they are very expensive and gained by order with different qualification, and different maturation rates. Objective: the aim of study to evaluate the efficacy of Origio IVM medium used in in vitro maturation of germinal vesicles gained during ICSI cycles. Patients and methods: 81 oocytes in germinal vesicle stage involved in in vitro maturation (IVM) program with Origio medium, they represent 35 cases (couples) that underwent IVF-ICSI operation, gained from them, (18) of them excluded because they are morphologically abnormal, the rest (63) 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 (63) GV incubation for 24 hours (7) of them remain in GV stage, (13) of them pass to M1 maturation stage, while (33) oocytes passes to M2 stage of maturation, the rest (10) they were atretic. (7) oocytes in GV stage represent (11,11 %) of total number, and (13) oocytes in M1 stage represent (20,63%), (33) oocytes in M2 stage represent (53 ,38%) while (10) was atretic represent (15,87%). The residual (7) oocytes GV and (13) 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 28.57 % of incubated GV, (3) of them converted to M1 stage which represent 48.86 % of them, and (1) of them transform to M2. and (1) become atretic stage

represent (14.29) for each of them respectively. For M1 stage oocytes: (4) of them remain in M1 stage represent (30,77%), (5) of them converted to M2 stage represent (38,46) % of them and the rest (4) become atretic represent (30,77%) (after incubation for another 24 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 (3,17 %) of total oocytes incubated, (8) oocytes were in M1 stage of maturation represent (12,70%), and (39) oocytes pass to M2 stage of maturation represent (61,90%), the rest 14 oocytes represent (22,22%) has been atretic. conclusion: Origio IVM medium give a good results in in vitro maturation process for human germinal vesicle with maturation rate reach to (61.90%) at 24-48 hours incubation period and can be used sufficiently with in ICSI cycles.

***Keywords*---**Maturation rate reach, IVM of human oocytes germinal, GV incubation.

Introduction

The term IVM refers to the maturation of the retrieved immature oocytes in the special culture environment (1).in which immature as opposed to mature oocytes are collected from the ovaries and allowed to mature in vitro prior to fertilization by either standard in vitro fertilization (IVF) on intracytoplasmic sperm injection (ICSI) (2)

During stimulated in vitro fertilization (IVF) cycle, up to 15-20 % of the recovered oocytes are immature ones which have poor fertilization capacity; however, the precise influencing factors are largely unknown (3). Reducing yield of mature oocytes is an another important aspect in stimulated IVF cycles because they usually have lower maturation capacity and decrease yield transferable embryos (4). In clinical practice, improving, developmental competence of these rescued oocytes *in vitro* would be useful for women who yield only immature oocytes or show total fertilization failure of *in vivo* matured oocytes in stimulated IVF cycles (5).

During stimulated cycles these immature oocytes fail to resume maturation or reach the mature (metaphase-II, MII) stage within 38 hours after administration of hCG. Immature oocytes may be retrieved at the germinal vesicle (GV); otherwise, they may undergo nuclear membrane breakdown without extrusion of the first polar body (metaphase I stage). Only mature oocytes are injected by ICSI on a regular basis, whereas immature oocytes are usually discarded (6). 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 (7).

Subject, Materials and Methods

81 oocytes in germinal vesicle (GV) stage introduced in in vitro maturation (IVM) program with origo (IVM) medium, they represent 35 cases (couples) that underwent IVF-ICSI operation, obtained from them, (18) of them excluded because they were morphologically abnormal, the rest (63) GV introduced in program, they incubated for 24 hours and reexamined again to assess their maturation, Antagonist hormonal protocol with different modification had been used with patients. Different types of drugs preparations were used for stimulation protocol.

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). And incubated for 24 hours and re-examine again to assess their maturity. The GV and M1 oocytes that remained re incubated for another 24hours and reexamined again to assess their maturity.

Results

1. GV incubation for 24 hours

(63) GV introduced in program, they incubated for 24 hours and examined to assess their maturation, they are founded in different maturation stages as the following:(7) of them remain in GV stage, (13) of them in M1 maturation stage, while (33) of them passes to M2 stage of maturation, the rest (10) they were atretic . (7) oocytes in GV stage represent (11,11 %) of total number, and 13 oocytes in M1 stage represent (20,63%), 33 oocytes in M2 stage represent (53 ,38%) while 10 was atretic represent (15,87%) as showed in figure (1)

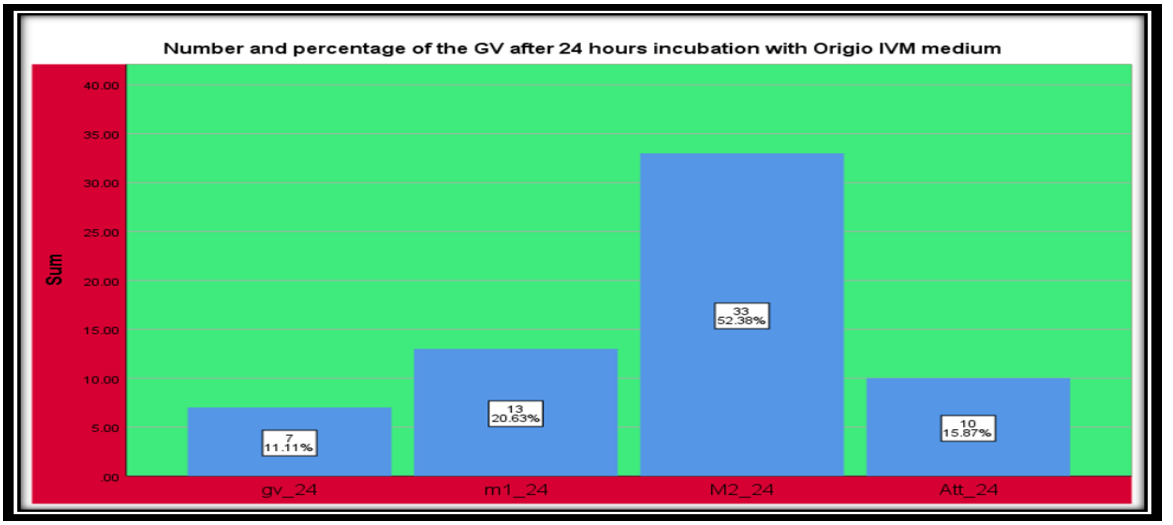

Figure (1): showed the percentage fate of GV after 24 hours' incubation with Origio medium.

4.2.2 residual GV and M1 incubated for another 24hours

(7) oocytes in GV stage and (13) 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 28.57 % of incubated GV, (3) of them converted to M1 stage which represent 48.86 % of them, and (1) of them transform to M2. and (1) become atretic stage represent 14.29% for each of them respectively. As showed in figure (2)

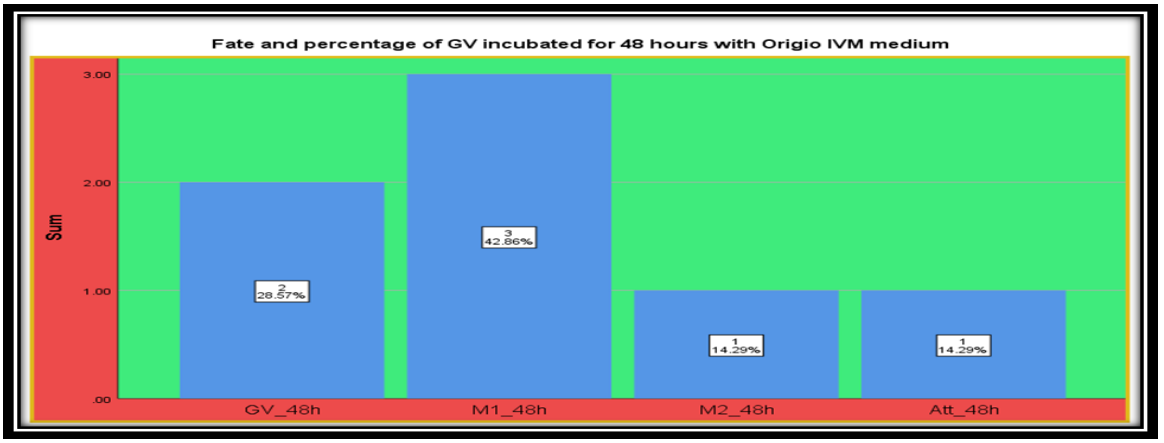

Figure (2): showed the fate and percentage of GV after 48 hours' incubation with fertilization medium.

For M1 stage oocytes: (4) of them remain in M1 stage represent 30,77%, (5) of them converted to M2 stage represent 38,46 % of them and the rest (4) become atretic represent 30,77% (after incubation for another 24 hours) as showed in figure (3)

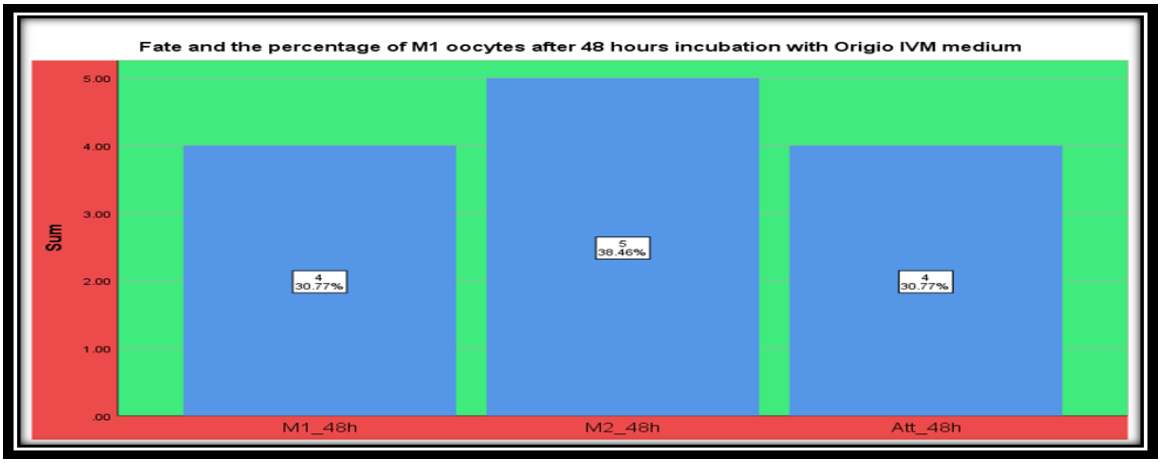

Figure (3): showed the number and the percentage of fate of M1 after 48 hours' incaution with Fertilization medium.

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 3,17 % of total oocytes incubated, (8) oocytes were in M1 stage of maturation represent 12,70%, and (39) oocytes pass to M2 stage of maturation represent 61,90%, the rest 14 oocytes represent 22,22% has been atretic.as showed in figure (4).

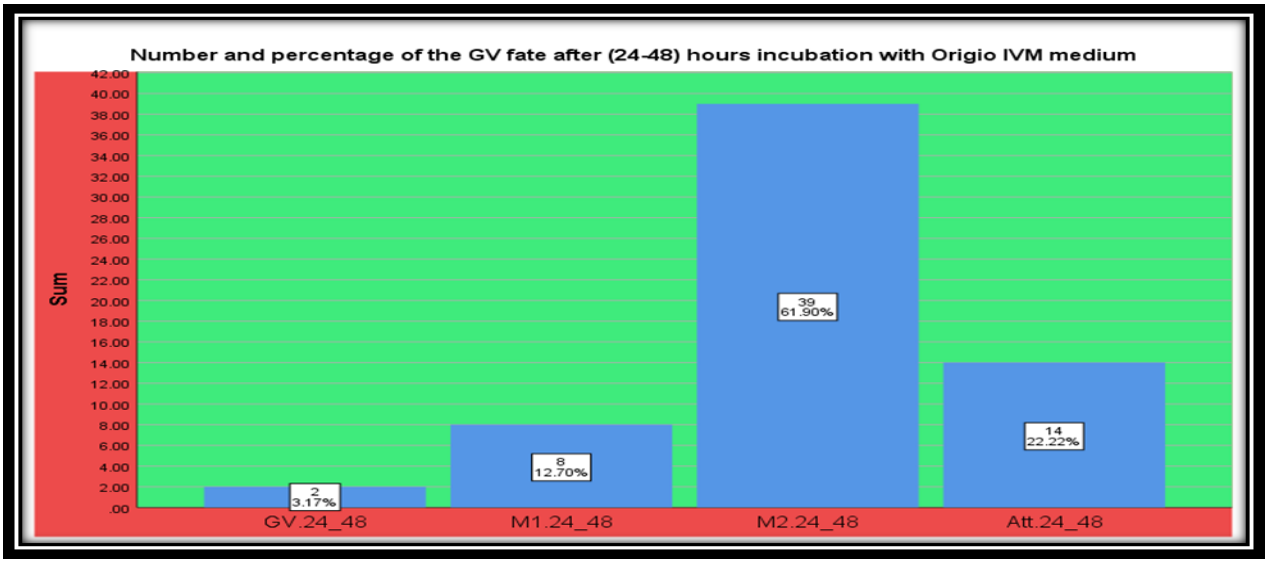

Figure (4): showed the number and percentage of the total GV fate after 24-48 hours' incubation with Fertilization medium

3. Comparison between different maturation stage and times

Results of comparison between different maturation stages and time of incubation for origio medium.as showed in fig (5) and table (1)

Paired Test	Mean	S.D	S.E. Mean	P .value	Sig.(P< 0.05)
GV/24h- GV/48h	.17857	.39002	.07371	.022	Sig

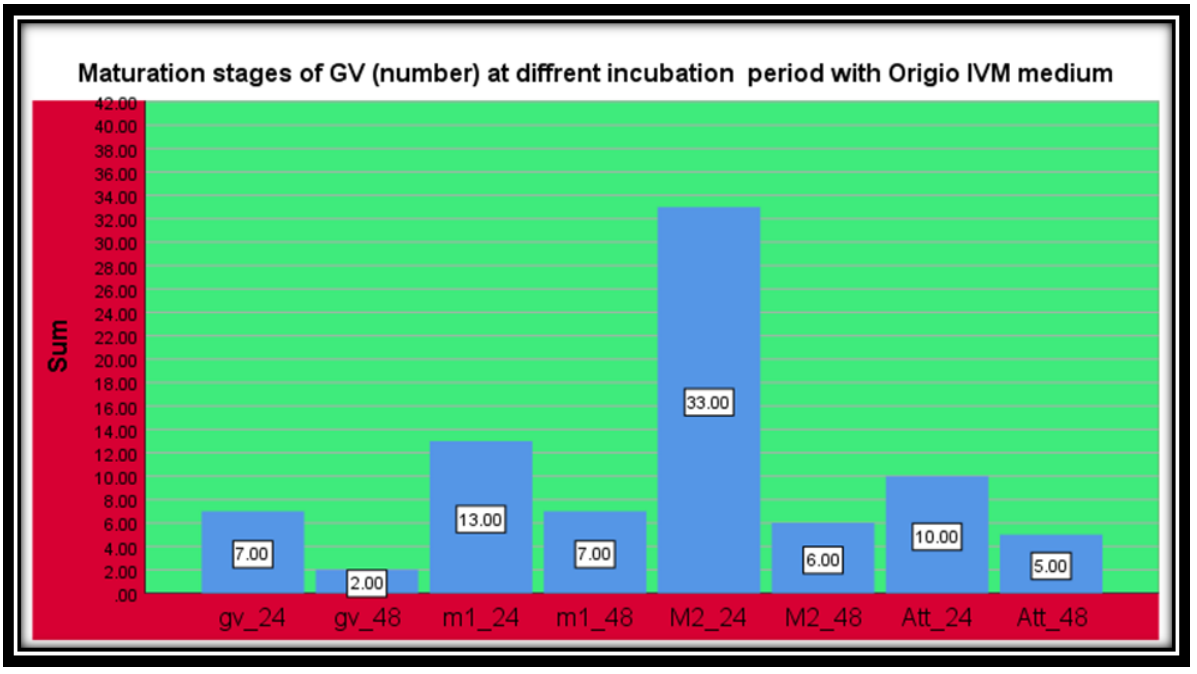

Figure (5): showed the different maturation stages of GV incubated for (24-48) hours incubation period with fertilization medium.

M1/24h- M1/24h	.21429	.56811	.10736	.056	Not Sig
M2/24h- M2/48h	.96429	.69293	.13095	.000	Sig
Att/24h- Att/48h	.17857	.86297	.16309	.283	Not Sig

There is a significant difference between residual GV that obtain from incubated for 24 hours and GV incubated for 48hours, (2) GV represent 28.57 % for 48 hours' incubation, while 7 GV represent 11.11% for 24hours incubation (p value < 0.05) for t- test.

And there is a significant difference in results of M2 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, 33oocytes converted to M2 in 24hours incubation represent 52.38% while in 48hours incubated period 6 oocytes converted to M2 stage of maturation represent 9.52%

Discussion

We found there is significant difference between maturation of GV 24 hours' incubation period and 48 hours' incubation period (p value < 0.05). The maturation rate of GV incubated for 24 hours' is better than 48 hours' incubation period in which (33) oocytes converted to M2 stage represent 52.38%. while (6) oocytes in 48 hours incubated period converted to M2 stage represent 9.52%.
These oocytes (GV) may receive a good stimulation to starting the maturation process. Stimulation protocol may activate the quiescent oocytes, so they are rapidly respond during incubation with medium. In addition to that the internal oocytes factor may play an important role in this results i.e. (cytoplasmic, nuclear, chromosomal, genetic factor)

also there is a significant difference between residual GV that obtain from incubated for 24 hours and GV incubated for 48 hours, (2) GV represent 28.57 % for 48 hours' incubation, while 7 GV represent 11.11% for 24 hours, incubation. (p value < 0.05) for t- test. The meaning of that; GV conversion to different maturation stage occurs during 24 hours of incubation. So there is no great benefit from extended incubation period to a long time .

The developmental capability of In Vitro matured oocytes is under influence of many factors, like as maturation environment, nature of medium and extracts. (8, 9, 10)

The ovum maturation course comprises the activation and inhibition of hormones growth factors and enzymes, leading to nuclear and cytoplasmic development. Nuclear maturation happens freely. The mechanical removal of the oocytes from covering follicular cells is able to prompting maturation of nucleus. The cytoplasmic maturation happens gradually. the ovum that reach to metaphase II earlier in an IVM program have good embryonic development. (11) In stimulated ICSI cycles, the unripe GV were better to incubated for 24–30 hours, and can be inseminated (ICSI) after expelling of the polar body. The maturation of denuded GV that deprived of cumulus cells in stimulated cycles occur during 24–26 hours(12) the best time for gaining a good number of mature oocytes was after 24 hours. An elongated time of GV incubation did not significantly rise the maturation rate. (13, 14. 15) .

Conclusion

Origio medium give a good results in in vitro maturation process for human germinal vesicle with maturation rate reach to (61.90%), and 24 hours incubation period is better time for incubation, and Origio medium can be used sufficiently with in ICSI cycles.

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