USING *IN-VITRO* MATURATION OF IMMATURE OOCYTES RETRIEVED FROM POOR RESPONDER PATIENTS TO IMPROVE PREGNANCY OUTCOMES IN SULAIMANI GOVERNMENT REGION, IRAQ

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Abstract

Background and Aim: This study is conducted to evaluate whether *in-vitro* maturation of immature oocytes retrieved from stimulated cycles can help to increase the number of embryos available for transfer and whether transfer of embryos derived from *in-vitro* matured oocytes would contribute to better clinical outcomes of poor responder patients in ovarian stimulated cycles.

PatientsandMethods: The present study was approved by the ethics committee in the College of Medicine,University of Sulaimani, Sulaimani, Kurdistan Government region, Iraq. Written informed consent was obtained, in which patients informed and understood to share the outcomes of their cycles for research purposes. The present study includes 92 poor responder cycles for intra cytoplasmic sperm injection. It comprises three and less mature oocytes (Metaphase II stage) with at least one immature oocyte (Metaphase I and Prophase I stage) which are retrieved after follicle aspiration and cycles are split into two groups control group (n=51), in which only mature oocytes are used, and *in-vitro* maturation (IVM) group (n=41)

where the immature oocytes are matured *in-vitro* and used, then the outcomes are compared. In *in-vitro* maturation group, oocytes are classified based on nuclear maturation status in to mature (Metaphase II) and immature oocytes (Metaphase I and Prophase I). The rates of *in-vitro* maturation, fertilization, and high-quality embryos are compared.

Results: Between the control group and *in-vitro* maturation group, no differences are observed as far as the implantation rate is concerned (12.5%, 12.5% respectively, $p > 0.05$), while the *in-vitro* maturation group has a higher number of transferred embryos than the control group (1.54±1.12, 1.1±0.9, respectively, $p<0.05$), a higher pregnancy rate (21.9%, 18.9%, respectively, $p<0.05$), with a lower embryo transfer cancellation rate (21.9%, 27.45% respectively, $p<0.05$). In *in-vitro* maturation group, immature oocytes have lower fertilization rate (40.47% versus 58.33%) with ($p <0.05$), higher high-quality embryos (64.7% versus 51.43%) with ($p< 0.05$) and no difference between Metaphase I and Prophase I in the rates of *in-vitro* maturation, fertilization and high-quality embryos.

Conclusion: The results show that human immature oocytes recovered from stimulated cycles can be matured, fertilized and developed *in-vitro* but they have lower fertilization rate than the *in-vivo* matured oocytes. Embryos derived from *in-vitro* matured oocytes may contribute to the better outcome in poor responder cycles.

Keywords: In-vitro maturation, immature oocytes, poor responder

Introduction

Since the momentous birth of Louise Brown in 1978, the focus of assisted reproductive technology (ART) has been on securing numerous mature oocytes prior to their removal from the ovary (Pellicer et al., 1989). This procedure allows optimal *in-vivo* oocyte maturation prior to their retrieval, challenging the laboratory to accomplish fertilization and early embryonic division before their transfer and possible implantation to the endometrial cavity. The past decades of refinements have been evolved with the oocyte retrieval process moving toward a more simplified, minimally invasive transvaginal ultrasound-guided aspiration combined with extremely technological advanced fertilization procedures, such as intracytoplasmic sperm injection (ICSI) (Russell et al., 1997). Laboratory culture techniques examine a variety of conditions including different media preparations with or without serum supplementation, different media enhancing additives, and the controversial use of co-culture techniques (Magli et al., 1996). Clinically, ovulation induction protocols focus on enhancing recruitment of the cohort of follicles and synchronizing their development by down-regulation of the central axis using gonadotrophin-releasing hormone (GnRH) agonists (

Meldrum et al., 1989). All of these factors have added to the ability to offer *in-vitro* fertilization (IVF) to numerous patients with multiple diagnoses with gradually improving clinical pregnancy rates. A process introduced several years ago in animal studies utilized *in-vitro* oocyte maturation as a supply of mature oocytes; In 1935, Pincus and Enzmann reported that immature rabbit oocytes removed from their natural ovarian environment are capable of undergoing spontaneous maturation and fertilization *in-vitro*(Pincus and Enzmann, 1935). Similar observations were later reported in human beings by Edwards in 1965. This practice was then introduced into human clinical trials during the early days of IVF (Edwards et al., 1965; Tsuji et al., 1985). This procedure has been successful in producing live born offspring in animals (Keskintepe et al., 1994), and in humans (Russell et al., 1997). The challenge with human *in-vitro* oocyte maturation is to provide optimal conditions for *in-vitro* maturation by mimicking the micro-endocrine environment of the developing follicle to enable the immature oocyte to achieve nuclear and cytoplasmic maturation. Once oocyte maturation *in-vitro* is completed, fertilization and culture conditionsmust also be optimal prior to the transfer of embryos to the uterus for the implantation to take place (Smith et al., 2000) Under controlled ovarian stimulation, some of the collected oocytes are approximately 20% immature (Smith et al., 2000). These oocytes are usually discarded due to the possibility of abnormal embryonic development, or an increased rate of abortion. However, in cases of poor responders and in patients with an unsynchronized cohort of follicles, where the presence of immature oocytes is frequent after stimulation (Smitz and Cortvrindt, 1999), the use of immature oocytes for IVF is important in order to increase the number of embryos obtained in each cycle. Based on the assumption that oocyte maturity is a prerequisite for obtaining normal fertilization, attempts have been made both in mammals and in human to mature germinal vesicle (GV) which is prophase I oocytes and metaphase I (MI) oocytes *in-vitro*(Huang et al., 1999).

Patients and methods

Study Design

The present study was approved by the ethics committee in the College of Medicine, University of Slemani, Slemani, Kurdistan Government Region, Iraq. Written informed consent was obtained, in which patients informed and understood to share the outcomes of their cycles for research purposes. The study included 92 poor-responder patients undergoing ICSI cycle, in which three and less M II oocytes and at least one immature oocyte (M I or P I oocyte) were retrieved after follicle aspiration in Dwarozh-IVF center between February 2010-July 2011.Cycles were divided into two groups, control group in which only mature oocytes are used

(control group, n= 51), and (IVM group, n= 41) the immature oocytes were also injected but after *in-vitro* maturation. The rates of embryo transfer cancellation (cycle with cancel transfer per total cycles), clinical pregnancy (presence of gestational sac with fetal heart beat visualized by ultrasound 4-6 weeks after embryo transfer, implantation (total number of gestational sacs divided by the total number of embryos transferred) and miscarriage (spontaneous loss of a pregnancy before 24 weeks of gestation) were compared. In IVM group oocytes are classified into mature (MII stage) oocytes and immature oocytes (MI and P I). Rates of *in-vitro* nuclear maturation (oocytes which complete meiosis I and enter metaphase of meiosis II per total immature oocytes were subjected to *in-vitro* maturation), fertilization (two pronuclei were seen in the oocyte after 16-18 hours of ICSI) and, high-quality embryos (either 4–6 cells on day 2 or 8–10 cells on day 3 of development, less than 15% fragmentation, symmetric blastomeres, absence of multinucleation, colourless cytoplasm with moderate granulation with no inclusions, absence of perivitelline space granularity and absence of zonapellucidadysmorphism) were evaluated.

Ovarian Stimulation

Ovarian stimulation had been done by gynecologist which started with baseline transvaginal ultrasonography (Siemens, Sonoline G20, Siemens Medical Solutions USA, Inc.) to document antral follicular count > 6 follicles and blood testing in the second day of the cycle for FSH (3.9-12 mIU/ml), E2 (18-147pg/ml), LH (1.5-8 mIU/ml), and P4 (≤ 0.25-0.54ng/ml) were performed with (miniVIDAS, bioMerieux, 06267 M, France) with using enzyme immuno assay competition method with a final florescent detection for E2, P4 and enzyme immuno assay sandwich method with final fluorescent detection for FSH and LH. In all patient short agonist protocol was used for induction of the ovaries which start GnRH agonist (Decapeptyl, Ferring GmbH, Germany) on day 2 and gonadotropins (Gonal-f, LaboroteriesSerono S.A, Switzerland; Fostimon, IBSA, Lugano 3, Suisse; Puregon, Schering-Plough, NV.Organon, Oss, Netherlands; Menegon, Ferring GmbH, Germany; and Merional, IBSA, Lugano 3, Suisse) Follow up of induction is started using transvaginal ultrasound (G20, Siemens, United States) and E2 and when ultrasound show ≥ 4 follicles with diameters of 16-17mm and E2 >500pg/ml, patients are administered a hCG (Choriomon, IBSA, Lugano 3, Suisse; Ovitrelle, IndustriaFarmacuticaSerono S.P.A, Bari, Italy). This medication contained the pregnancy hormone, hCG, which functions similarly to LH is an important hormone that helps in maturation of the ova, to be ready for fertilization, and stimulates ovulation (Holzer et al., 2006).

Oocyte Retrieval

Oocyte retrieval had been done by gynecologist 34-36 hours following hCG administration, Oocyte retrieval was performed by vaginal ultrasound- guided puncture using 16 gage 35cm double lumen aspiration needle (William A.Cook, Australia pty Ltd.) with negative pressure 20 mmHg under intravenous general anesthesia. Follicular fluid aspirated into tube containing aspiration media and heparin (Ferticult Aspiration, Fertipro N.V, Belgium), then the oocyte-cumulus complex was collected under stereo microscope (Nikon, SMZ 1500, Tokyo, Japan) and washed with flushing media (Ferticult flushing, Fertipro N.V, Belgium) then placed inside IVF media (Ferticult IVF, Fertipro N.V, Belgium)in incubator 37 ˚C and 6% CO2 for 1-2 hours.

Denudation of The Oocytes

Removal of the surrounding cumulus cells is accomplished by a combined enzymatic exposure to hyaluronidase enzyme in HEPES-buffered medium containing 80 IU/ml (Hyase, Fertipro N.V, Belgium) and mechanical treatment. The oocytes are repeatedly aspirated stepwise through a pasteur pipettes with an inner diameter of 290µm and 135µm (Ez-strip, Research Instruments Ltd, United Kingdom); the procedure was carried out under a stereo dissecting microscope (Nikon, SMz1500, Tokyo, Japan). Each oocyte was then examined under the microscope to assess the maturation stage and its integrity. Metaphase II (MII) was assessed according to the absence of the germinal vesicle (GV) and the presence of an extruded polar body see micrograph (3.1) metaphase I (MI) was assessed according to the absence of both (GV) and extruded polar body see micrograph (3.2), and prophase I (PI) when (GV) was obvious in the absence of extruded polar body see micrograph (3.3) (Dickmann, 2005).

***In-Vitro* Maturation**

Immature oocytes of patients (MI and PI) were placed in SAGE IVM medium (Cooper Surgical, Trumbull, CT), which contain basic media component (sodium chloride, potassium chloride, sodium bicarbonate, sodium pyruvate, glucose, phenol red and gentamycin), essential amino acids, non-essential amino acids, vitamins, and plasma protein fractions with the addition of 75 mlU/mL of FSH and 75 mlU/mL of LH according to the manufacturer's recommendation. The time at which these immature oocytes were placed in IVM media considered as time zero for IVM. After approximately 24 h of incubation in IVM medium, the oocytes were re-evaluated; those oocytes that achieved maturation as mentioned earlier underwent second day injection with new prepared semen sample, while those which remained immature were discarded.

Sperm Preparation

The semen collected by masturbation in all cycles; then allowed to be liquefy for 15-30 minutes. After liquefaction analysis was done with Makler chamber (PolymedcoInc., Yorktown, NY) using phase contrast microscope (Olympus, BX41, Tokyo, Japan) according to WHO criteria. The semen was prepared using colloidal silica density gradient centrifugation (Sil-select Stock, Fertipro N.V, Belgium)as recommended then kept inside incubator 37˚C until the time of ICSI.

ICSI Procedure

A drop of prepared sperms were placed in a ICSI dish (Nunc A/S, Denmark), sperms are aspirated with the injection pipette and transferred to 4 µl droplet of polyvinylpyrrolidone solution which is a viscous synthetic polymers (PVP, Fertipro N.V, Belgium) for immobilization.(Brooks et al., 2000).Active spermatozoa must first be immobilized; this is accomplished by gently lowering the tip of the pipette so as to compress the mid region of the sperm flagellum against the bottom of the dish, then it is drawn into the injection pipette from its tail (Brooks et al., 2000), meanwhile the oocytes were placed in 5 µl droplets of Flushing medium which was covered by lightweight mineral oil (Ferticult Mineral oil, Fertipro N.V, Belgium) in order to maintain stable temperature, osmolality and pH (Miller et al.1994).ICSI was done as in (Nagy et al., 1995), and was carried out on the heated stage (37°C) of an inverted microscope (Integra Ti, R.I., Olympus, IX51/IX70, Tokyo, Japan) 40 h after HCG trigger for MII-stage retrieved oocytes and 64 h after HCG trigger for immature oocytes that had undergone nuclear maturation. After injection, the oocytes were washed and stored in 25 µl micro drops of culture medium under oil in a culture dish. They were kept in an incubator at 37°C in an atmosphere of 6% CO_2 for 16-18 hours.

Assessment of Fertilization, Embryo Quality and Embryo Transfer

At 16–18 h after microinjection, the oocytes were checked for survival and fertilization. The numbers and aspects of polar bodies and pronuclei are recorded. The criteria for normal fertilization are the presence of two clearly visible pronuclei. Embryo cleavage and the quality are evaluated 2 days after ICSI (Nagy et al., 1994).

Embryo quality was evaluated under an inverted microscope. The following parameters were recorded: (i) the number of blastomeres; (ii) the fragmentation percentage; (iii) variation in blastomere symmetry; (iv) the presence of multinucleation; and (v) defects in the zonapellucida and the cytoplasm. High-quality (grade A) embryos were defined as those having all of the following characteristics: either 4–6 cells on day 2 or 8–10 cells on day 3 of development, less than 15% fragmentation, symmetric blastomeres,

absence of multinucleation, colourless cytoplasm with moderate granulation with no inclusions, absence of perivitelline space granularity and absence of zonapellucidadysmorphism. Embryos lacking any of the above characteristics are considered as low quality (de Almeida et al., 2010).

For each couple, one to three embryos were transferred for female age ≤ 35 years old and more than three embryos for female age > 35 years old, if available. Embryo transfer was cancelled when there were no embryos available, when patients in each group had complete fertilization failure or the embryos failed to divide or embryo arrested division. In the IVM group, priority for transfer was given for embryos derived from MII oocytes. Embryo transfer was performed on day 2 or day 3, with the use of a Gynetics catheter (Gynetics Medical Products N.V, Lommel, Belgium), then cycles followed by serum B-HCG and Ultrasonography. Micrograph was taken with video camera 3mega pixel (WAT-221S, Olympus, Tokyo, Japan) loaded over the inverted microscope connected to Cronus software (Research Instruments Ltd, United Kingdom)

Assisted Hatching

For increasing implantation assisted hatching (Cohen et al., 1990) which is a procedure done with using non-contact laser, type (Saturn 3, Research Instrument, UK) with 1480nm/ 400mW solid state diode laser. Pulse length range 0.1 – 2.0ms / 100-2000 microseconds, which cause thermolysis to the zonapellucida matrix and thinning of the zonapellucida. Assisted hatching is done for all patients (Brooks et al., 2000),

Statistical Analysis

Results were expressed as mean ± standard deviation (SD) for numeric variables and percentages for categorical variables. Mean values were compared by Students t-test, and proportions were compared by chi-squared test. The results were considered to be significant at the ($P < 0.05$). Data analysis was carried out using the statistical analysis program (Statistical Package for Social Science SPSS) version 17.

Results

General Characteristics

From 92 cycles included in this study, Female-factor infertility was found in 29 (31.52%) of the cycles, male-factor infertility was present in 24 (26.08%) of the cycles, both-factor were responsible in 31(33.69%) of cycles, and unknown cause in 8 (8.7%) of them. Primary infertility was found in 73(79.35%) of the cycles and 13(14.13%) of them were their second attempt. The results showed no significant difference in age of female patients and years of infertility between IVM and control groups which was

(33.22±6.5, 33.63±6.2, p value > 0.05), (8.6±6, 6.6±3.3, P value > 0.05) respectively, but significant mean age for poor responder which was 33 years old.No significant difference in the number of retrieved oocytes which was (5.63±3.7, 4.73±2.1, P value < 0.05), were seen but the percentage of collected immature oocyte was significantly higher in IVM group than control group which was (65.68%, 43.98%, P <0.05) respectively as shown in table (3.1).

General characteristics	Control group N= 51	IVM group N= 41	P value
Female age	33.22 ± 6.5	33.63 ± 6.2	0.754
Year of infertility	8.61 ± 6.1	6.65 ± 3.33	0.070
Induction duration	11.76 ± 1.2	11.54 ± 1.5	0.769
oocytes retrieved	4.73 ± 2.1	5.63 ± 3.7	0.394
Immature retrieved oocytes	43.98%	65.68%	0.001
Values are mean± SD or % SD= standard deviation			

Table (3.1): General Characteristics of Control and IVM Groups

For the hormones in the second day of the cycle, the result showed significant lower E2 level in control group than the IVM group which was (38.3±15.3, 45.97±28.6, P value <0.05) and no significant difference in FSH and LH levels which were (7.1±4, 7.4±3.1, P value < 0.05) and (3.04±1.4, 3.3±1.8, P value < 0.05) while the level of P4 showed significant differences which was higher in the IVM group than the control group (4.9±5.35, 1.6±4.4, P value< 0.05) as shown in table(3.2). The levels of E2 in the day of HCG injection were significantly higher in the IVM group (1209.43±693.3) than the control group (1047.93±658.9) with (P value < 0.05) as shown in table (3.2).

Nuclear Maturation Status and *In-vitro* Maturation:

In IVM group, the overall *in-vitro* matured oocytes were compared to the *in-vivo* matured oocytes and the results indicate that the percentage of fertilized oocytes were significantly higher in *in-vivo* matured oocytes than *in-vitro* matured oocytes, which was (58.33%, 40.47% respectively; P < 0.05), but high-quality embryos was significantly higher in *in-vitro* matured oocytes than the *in-vivo* matured oocytes, which was (64.7%, 51.43% respectively; P < 0.05) as shown in table(3.3).

Table (3.3): Compared Results Between *In-vivo* and *In-vitro* Matured Oocytes Used In IVM Group.

	In-vitro matured oocytes (PI and MI). N=148	In vivo matured oocytes (MII). N= 64	P value
In-vitro matured	93 (62.84%)	-------	
injected	84 (90.32%)	60 (93.75%)	0.713
Fertilized	34 (40.47%)	35 (58.33%)	0.004
High-quality embryos	22 (64.7%)	18 (51.43%)	0.005
PI= prophase I, MI= metaphase I, MII= metaphase II			

In IVM group 231 oocytes were retrieved, from these oocytes 64 (27.7%) were in MII stage, 78 (33.76%) were in MI stage and 70 (30.3%) were PI stage; both PI and MI were subjected to *in-vitro* maturation. Results demonstrate that 42 (60%) of PI oocytes completed maturation *in-vitro* and 51 (65.38%) MI matured *in-vitro*, 36 (85.7%) and 48 (94.11%) respectively were injected from each and the difference in fertilization and high-quality embryos rates between PI and MI; statistically, the difference are not significant as shown in table (3.4).

Table (3.4): Compared Results Between Immature Oocytes Prophase I and Metaphase I Using in the IVM Group.

	PI N= 70	MI N= 78	P value
In-vitro matured	42/70 (60%)	51/78 (65.38%)	4.99 NS
Injected	36/42 (85.7%)	48/51(94.4%)	0.062 NS
Fertilized	13/36 (36.1%)	21/48 (43.75%)	0.369 NS
High-quality embryos	9/13 (69.23%)	13/21 (61.9%)	0.664 NS
PI= prophase I, MI= metaphase I			

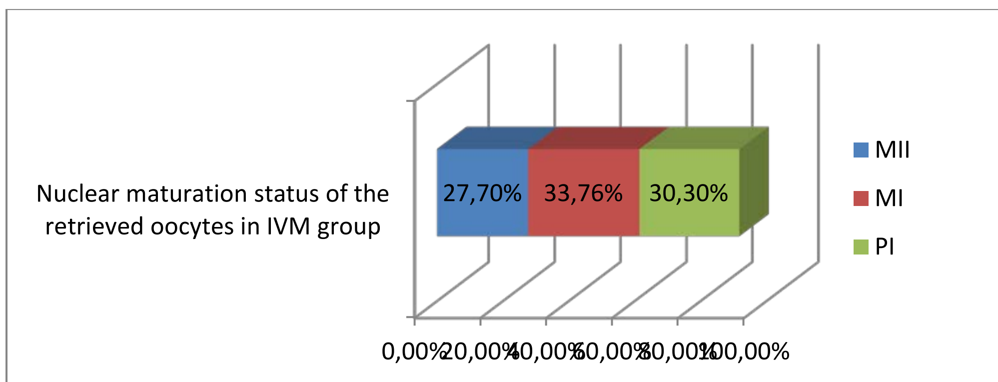


Figure (3.1) Nuclear Maturation Status of Retrieved Oocytes in IVM Group

Fertilization and Embryo Quality

ICSI was performed in 259 oocytes in both control and IVM groups. In IVM group, 144 ICSI was performed, 84(58.33%) of them were immature oocytes. From these, 36(25%) of them were derived from PI stage and 48(33.33%) of them were from MI stage. The results indicate that fertilization rates were significantly higher for *in-vivo* matured oocytes when compared to *in-vitro* matured oocytes(58.33%, 40.47% respectively; P < 0.05) and percentage of high-quality embryos derived from *in-vitro* matured oocytes as micrograph:(3.6;3.10) (are higher than the *in-vivo* matured oocyte (64.7%, 51.43% respectively; P > 0.05) as shown in table (3.3).

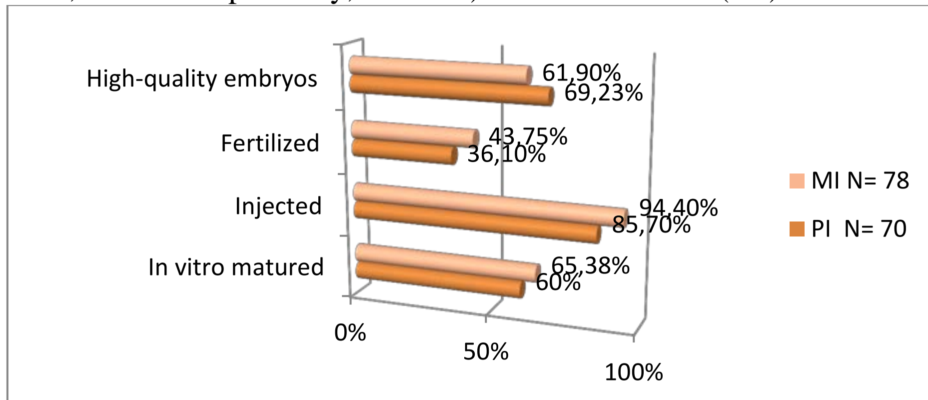


Figure (3.2) Compared Result Between Metaphase I and Prophase I Oocytes in IVM Group

Embryo Transfer and Clinical Outcome

Embryo transfer was performed at day 2 and/or day 3, 14(43.8%) of IVM group and 24(64.9%) of control group with assisted hatching. The number of transferred embryos are significantly higher in IVM group than the control group (1.54 ± 1.12, 1.10 ± 0.88 respectively; P < 0.05).

Table (3.5): Comparing number of embryo obtained between control and IVM group

	Control group	IVM group
No. of patients	51	41
No. of retrieved oocytes	241	231
Mature oocytes	115	64
Immature oocytes	106	148
No. of injected oocytes	115	144 (60 from in-vivo mature and 84 from in-vitro matured oocytes)
No. of embryo developed	61	68 (13 from in-vivo mature and 55 from in-vitro matured)
No. of transferred embryos	56	64
Mean ± SD of transferred embryo	1.1±8.8	1.5±1.12 (P value< 0.05)

No difference was observed in implantation rate which was 12.5% for both groups. Pregnancy rate was significantly higher in IVM group than the control group which was (21.9%, 18.9% respectively; P < 0.05). The percentage of embryo transfer cancellation was significantly higher in the control group than the IVM group which was (27.45%, 21.9%; P < 0.05) as shown in table (3.6).In IVM group, 32 (78.04 %) cycles had transfer out of the 41 cycles, in which 4 (12.5%) of the embryos were derived from only *in-vivo* matured oocytes, 16 (50%) embryos were derived from both *in-vivo* and *in-vitro* matured oocytes, and 12 (37.5%) embryos were derived from only *in-vitro* matured oocytes with implantation rate (7.7%).

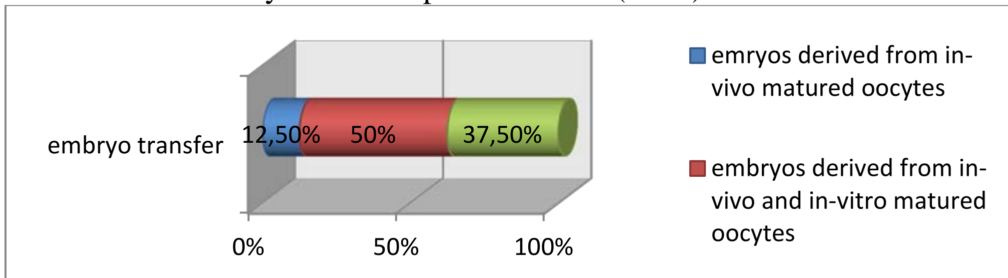


Figure (3.3) Type of Embryos Transferred in IVM Group

From 7(21.9 %) clinical pregnancy, 4(12.5%) of them ended in abortion before 8 weeks and 3(9.37%) ongoing pregnancy; two singleton and one twin pregnancy ended with labour of four healthy babies.In control group, 37(72.55%) cycles had transfer out of 51 cycles, in which from 7(18.92%) clinical pregnancies again 4(10.81%) ended in abortion before 8 weeks and 3(8.1%) ongoing pregnancies with three singleton ended with labour of three healthy babies. The percentage of abortion and ongoing pregnancies were compared between both groups and show no significant difference with p value (0.11, 0.22) respectively.

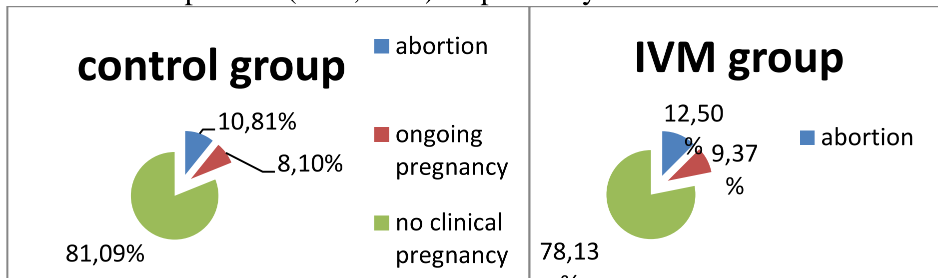
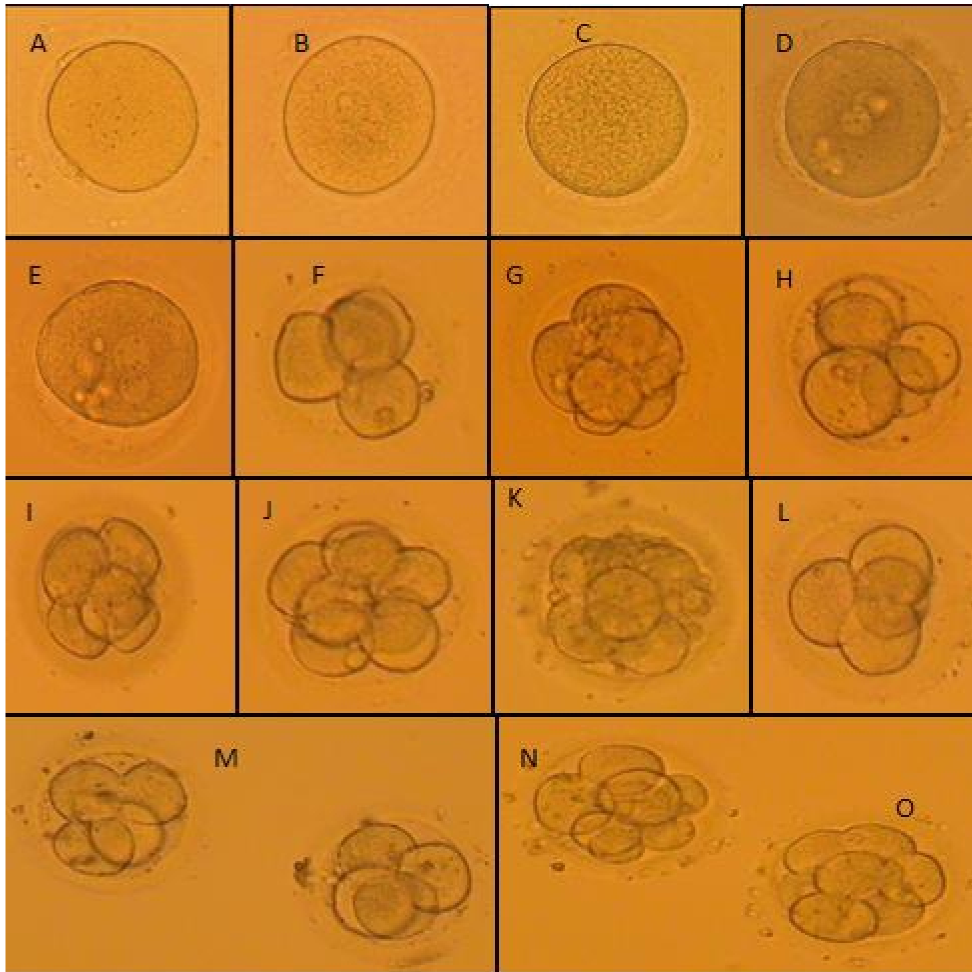


Figure (3.4) Percentage of Abortion and Ongoing Pregnancy in Control and IVM Group



Micrograph(1): Stages of oocyte maturation, fertilized oocytes and embryo development.(A)Metaphase II Oocytes (400X), (B) Prophase I Oocyte (400x), (C) Metaphase I Oocyte (400x) (D) Fertilized In-vivo Matured Oocyte (400x), (E) Fertilized In-vitro Matured Oocyte (400x), (F) Day 2 High-quality Embryo Derived from In-vitro Matured Oocyte (200x), (G) Day 2 Low-quality Embryo Derived from In-vitro Matured Oocytes (200x), (H) Day 2 Low-quality Embryo Derived from In-vitro Matured Oocytes (200x), (I) Day 3 Low-quality Embryo Derived from In-vitro Matured Oocyte (200x), (J) Day 3 High-quality Embryo Derived from In-vitro Matured Oocyte (200x), (K) Day 3 Low-quality Embryo Derived from In-vitro Matured Oocyte (200x), (L) Day 2 High-quality Embryo Derived from In-vivo Matured Oocyte (200x), (M) The Low-quality Embryos Derived from In-vivo Matured Oocytes in Day 2 (200x), (N) Day3 Low-quality Derived from In-vivo Matured Oocyte (200x), (O) Day3 High-quality Derived from In-vivo Matured Oocyte (200x).

Discussion

In a stimulated cycle, an administered pharmacological dose of gonadotropin creates a supra-physiologic hormonal environment and induces simultaneous growth of a cohort of large and small follicles. The heterogeneity of the oocyte population at the time of HCG administration leads to retrieval of oocytes at different stage of maturation.The present study is designed to assess the developmental potential and clinical implications of IVM of immature oocytes retrieved from controlled ovarian stimulation for IVF/ICSI cycles, specifically investigating 24 hours of IVM with subsequent second day injection of matured oocytes. The overall retrieved oocytes were 472,from which 37.92% were M II stage and 27.33% were M I stage and 26.31% were in P I. The percentage of retrieved mature oocytes was lower than the results reported by other studies(Kim et al. 2000; Patricial et al. 2003; Strassburger et al. 2004). One explanation for this could be thatthe patients included were poor responders. Various studies have revealed the benefit of 24 hours IVM of immature oocytes from stimulated cycles (Patricial et al., 2003; Reichman et al., 2010; de Almeida et al., 2010). The present study showed that the rate of *in-vitro* maturation of M I stage oocytes were 65.38% which was lower than the rates of some other studies (Reichman et al., 2010, de Almeida et al., 2010) in which their results showed (78.2% and 75.8%, respectively) even higher rates than the results of (Patricial et al., 2003) which was (63.2%) reported. In the same way, the rate of matured P I stage oocytes were 60% which is higher than the results of (Patricial et al., 2003; Riechman et al., 2010) which was (38.6% and 35.1%) respectively, and lower than the (72.7% and 70%) which was found by (de Almeida et al., 2010; Farsi et al., 2011). *In-vivo* oocyte meiotic resumption requires intimate paracrine and autocrine signaling between the oocyte and its surrounding granulosa and cumulus cells (Sun et al., 2009). ICSI involves the removal of cumulus and corona cells shortly after oocyte retrieval in order to make a determination of nuclear maturity, as it is difficult to identify nuclear maturity under the microscope with the cumulus –oocyte-complex intact (Cha and Chian, 1998). Removing cumulus cells, however, disrupts signaling pathways between those oocyte discovered to be immature and their surrounding cells. Cumulus cells share an integral role in meiotic arrest via gap-junction mediated signaling and maintenance of oocyte cAMP levels, while also exerting stimulatory effects on nuclear and cytoplasmic maturation, as evidenced by higher rates of maturation (Goud et al., 1998; Sun et al., 2009). In order to analyze the literature regarding IVM of immature oocytes, it is critical to note whether cumulus complexes are left intact or are stripped prior to placement in culture media. In this study, cumulus were stripped partially to confirm polar body emission and to perform ICSI subsequently.The present study showed lower fertilization

rates of *in-vitro* matured oocytes than the *in-vivo* matured oocytes which was similar to the previous studies (De Vose et al., 1999; Kim et al., 2000; Aycan et al., 2003; Behr et al., 2004; Strassburger et al., 2004; de Almeida et al., 2010; Riechman et al., 2010).The reduced fertilization rate of *in-vitro* matured oocytes can be explained by the cytoplasmic immaturity of these oocytes in contrast to their nuclear maturation. (Eppig, 1996; Heikinheimo and Gibbons, 1998; Trounson et al., 2001; Combelles et al., 2002).In comparing the rates of high-quality embryos between *in-vivo* matured oocytes and *in-vitro* matured oocytes the results of this study show that high-quality embryos are higher significantly in the *in-vitro* matured oocytes which differs from the results of others studies (Strassburger et al., 2004; Farsi et al., 2011) that show no significant difference between *in-vivo* matured and *in-vitro* matured oocytes in regard to the number of blastomeres, similarly, (de Vose et al., 1999) found no significant difference in excellent and fair quality embryos but significantly higher good quality embryos in *in-vivo* matured oocytes, while a study by (de Almeida et al., 2010) report that the development of high-quality embryos are significantly higher in the *in-vivo* matured oocytes.In the present study a comparison were made between the implantation rate, and the clinical pregnancy rate between control and IVM groups in order to evaluate the usefulness of rescued IVM of immature oocytes retrieved from stimulated cycles. It was found that the IVM of immature oocytes showed lower fertilization rate but significant increases in the number of embryos available for transfer (1.54 ± 1.12 versus 1.1 ± 0.88), decreases in embryo transfer cancellation (27.45% versus 21.9%) as in a study conducted by (de Almeida et al., 2010) which showed (2.35 versus 1.87) and (14.5% versus 6.36%) respectively. This study also showed increases in clinical pregnancy rate (21.9% versus 18.9%) but no significant difference is reported in both studies by (de Almeida et al., 2010; Reichman et al., 2010), and no significant difference in the implantation rate as this study showed to be (12.5%) for both group which was compatible with (de Almeida et al., 2010) results. In 12(37.5%) of IVM cycles, embryos derived from *in-vitro* matured oocytes were only available for transfer and their implantation rate was (7.7%), which only two patients conceived but pregnancy terminated with abortion , and this result is higher than (4.7%) reported by (de Almeida et al., 2010), (4%) as reported by (Shu et al., 2007) and (zero) by (Reichman et al., 2010) and lower than the results of (Liu et al., 2003) which was 2 singleton and one twin pregnancy when only embryos derived from *in-vitro* matured oocytes were transferred in eight cycles.The results showed that human immature oocytes recovered from stimulated cycles can be matured, fertilized and developed *in-vitro* but they have lower fertilization rate than the *in-vivo* matured oocytes. Embryos derived from *in-*

vitro matured oocytes may contribute to the better outcome in poor responder cycles.

Conclusion

The results show that human immature oocytes recovered from stimulated cycles can be matured, fertilized and developed *in-vitro* but they have lower fertilization rate than the *in-vivo* matured oocytes. Embryos derived from *in-vitro* matured oocytes may contribute to the better outcome in poor responder cycles.

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