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Original Article

Natural-cycle *in vitro* fertilization (IVF) combined with *in vitro* maturation in infertile patients with polycystic ovarian syndrome (PCOS) requiring IVF

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ABSTRACT

Objective: In this study, we report an experience of 59 natural-cycle IVF combined with *in vitro* oocyte maturation (IVF/M) cycles in patients with PCOS requiring IVF recruited based on limitations to afford a conventional IVF treatment in a 9-years period. Results of IVF/M were compared with 164 cycles of IVF in PCOS patients.

Material and methods: In IVF/M cycles only hCG priming was used before oocyte recovery, with *in vitro* maturation of immature oocytes in a commercial medium. In conventional IVF group, recombinant FSH (rFSH) and GnRH agonist/antagonist for ovarian stimulation were used. In both groups, fertilization was achieved by intracytoplasmic sperm injection (ICSI) of mature oocytes and fresh embryos transferred at day 2 or day 3.

Results: In all IVF/M cycles oocytes and transferable quality embryos were obtained, only in 6 IVF/M cycles mature oocytes were obtained at oocyte capture day. Clinical pregnancy rate per cycle was 39.0% vs 53.6% ($p = 0.0682$) and delivery rate per cycle was 30.5% vs 42.6% ($p = 0.1209$) in IVF/M and conventional IVF respectively. Patients with ovarian hyperstimulation syndrome (OHSS) were 0% in IVF/M vs 6.7% in conventional IVF ($p = 0.0399$).

Conclusion: Our experience in a private clinic in Mexico suggests that IVF/M can be a useful initial strategy to treat PCOS patients requiring IVF with comparable delivery rates to conventional IVF and a decreased risk of ovary hyperstimulation. IVF/M may be indicated to patients with limited resources paying without insurance for their infertility treatment.

Introduction

In vitro maturation (IVM) is an assisted reproduction technique (ART) with an interesting evolution. Since the report of the first pregnancy in the 90's [1], IVM was proposed as a technique with a lot of potential and gained popularity in infertility clinics. This initial enthusiasm has been lost because of reduced pregnancy rates of IVM when is compared to routine IVF [2]. Currently, few groups are active in clinical and basic research. Clinical trials are lacking and there are many questions in relation to laboratory procedures: how to optimize maturation rate and embryo development? which culture media to use? Also, we still have clinical uncertainties such as the importance of priming with FSH or hCG, the effect of lack of experience during immature oocyte pick up, the feasibility of blastocyst culture and the precise clinical indications for IVM [3].

A review of literature confirmed that IVM is inferior to conventional stimulation in relation to pregnancy and take-home baby rates [3]. This probably supports the apparent "abandon" of IVM during the last years and the resistance of IVF clinics worldwide to include IVM in their routine procedures. However, the movement of "mild approaches" and patient friendly-IVF are considering IVM as useful tool [4]. IVM can offer higher pregnancy rates than natural cycles and both natural cycles combined with IVM or IVM practically eliminate the risk of ovarian hyperstimulation syndrome (OHSS).

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Other attractive benefit is the reduction in cost of procedures which is the main limitation for access to ART in countries where IVF is not covered by health insurance or by the government [4].

In relation to safety, apparently IVM does not generate an increased risk of malformations or chromosomal abnormalities [3]. Currently more than 5000 births are estimated from IVM worldwide [4]. A review of 1421 babies born by IVM indicated no major risks, reassuring in relation to safety and clinical potential of IVM [5].

In this report, we describe our clinical experience and obstetrical outcome of IVF/M cycles versus routine IVF offered to PCOS patients requiring IVF in a private infertility clinic in Mexico.

Material and methods

Patients

We began to offer IVM as routine procedure in 2007. IVM is routinely offered to all patients in our center with an antral follicle count (AFC) > 15 who requires IVF and cannot afford it. The local review board approved the procedure and present report. All couples provided informed consent for the procedures. Consecutive IVF/M cycles to PCOS patients requiring IVF because of several indications without enough economic resources to perform IVF from June 2007 to December 2016 were included. For compare the results 164 cycles of conventional IVF of patients with PCOS treated during the same period were included.

All patients had a full infertility workup, including semen analysis, basal pelvic ultrasound, ovarian hormones, hysterosalpingography, hysteroscopy and serological test for HIV, hepatitis B and hepatitis C. The diagnosis of PCOS was established according to the revised Rotterdam ESHRE/ASRM criteria [6].

Ovary stimulation for IVF cycles

Patients in IVF group were stimulated using up to 150 U rFSH per day (Gonal-F, Serono) and GnRH antagonist (cetrorelix acetate) 0.25 mg per day (Cetrotide, Serono) or 0.1 mg of triptoreline (Gonapeptyl daily, Ferring Pharmaceuticals) with triggering when a follicle reached 18 mm using 5000 hCG (Choragon, Ferring Pharmaceuticals Mexico) or 250 μg of rhCG (Ovidrel, Merck Serono).

In-vitro maturation

A transvaginal ultrasound scan was performed to all patients enrolled on day 2 or 3 of their menstrual cycle (natural or induced) to asses AFC. The number of antral follicles (>2 mm in diameter) in both ovaries was recorded. No FSH priming was used. On day 7 another trans-vaginal ultrasound scan was performed, then daily or on alternate days thereafter as required. When at least one follicle reached 12 mm and/or endometrial thickness was ≥7 mm patients received a single IM injection of 10,000 hCG (Choragon, Ferring Pharmaceuticals Mexico) or 250 μg of rhCG (Ovidrel, Merck Serono). 37 h after hCG injection oocyte retrieval was performed under patient IV sedation using a 17G x 20 single lumen needle (Kitazato Oocyte Pickup Needle) and vacuum pressure of 50 mmHg. Aspirated fluid was collected in 10 mL round tubes containing a 0.9% NaCl solution supplemented with 2U/mL of sodium heparin.

Retrieved cumulus oocytes complexes were evaluated with an inverted microscope using the sliding method [7]. If no germinal vesicle (GV) was observed in the oocyte cytoplasm, cumulus cells were removed using hyaluronidase (InVitroCare) and mechanical pipetting for reassessment of maturity after denuding. When mature (metaphase II) oocytes were found on the collection day they were inseminated by ICSI. Immature oocytes (GV or metaphase I oocytes) were cultured in IVM media (SAGE) supplemented with 0.75 IU/mL of rFSH and 0.75 IU/mL of rLH. After 24 h of culture oocytes were examined, if oocytes remained at GV or MI stage those were cultured in the same medium and re-evaluated 24 h later. Oocytes in metaphase II were injected with spermatozoa obtained by gradient centrifugation (Pure Sperm) combined with a swim-up procedure. Injected oocytes were transferred to Global medium (LifeGlobal) covered with paraffin oil (LifeGlobal) and incubated at 37 °C in 6.5% CO_2. After 18 h, fertilization was evaluated by the presence of 2 pronuclei and 2 polar bodies. Embryos were culture in 20 μl droplets of Global medium at 37 °C in 6.5% CO2, according to standard procedures and embryo transfers performed on day 2 or 3 of embryo culture.

Endometrial preparation

Patients started oral estradiol valerate (EV) (Primogyn, Bayer Mexico) at the day of oocyte retrieval. Dosage was individually calculated depending upon endometrial thickness: < 7 mm, patients received 10 mg daily and if it was ≥7 mm a 6 mg dose was administered. Luteal support was provided with 50 mg/day of IM progesterone (Progesterone injection USP, Hikma Farmaceutica, Portugal) starting on the day of ICSI and continued along with EV, until 12 weeks of gestation, if patient was pregnant. Embryo transfer was performed using an Ultrasoft Frydman catheter set (Laboratorie CCD, France) with echogenic guide.

Statistical analysis

Statistical analysis was performed in GraphPad Prism 5 software. Proportions were evaluated by Exact Fisher test with two tailed p-values. Continuous variables are presented in tables as mean ± standard deviation and t-test were used for hypothesis testing using two tailed p-values.

Results

A total of 59 patients with PCO requiring IVF were enrolled to perform IVM as ART. All of them had a PCOS diagnosis and accepted to perform an IVF/M because reduction in cost of the procedure. This cohort was compared with 164 PCOS patients in conventional IVF cycles. Basal characteristics of both groups are show in Table 1, no significant differences were found on both groups. Indications for IVF in IVM/F vs IVF groups were tubal infertility (32.2% vs 29.3%), male factor (28.8% vs 31.7%), failure to conceive after 3 cycles of intrauterine insemination (25.4% vs 28%) and unexplained infertility (13.5% vs 11%) (Table 1).

We obtained a media of 16.9 ± 9.4 oocytes vs 18 ± 9.8 (p = 0.4557) per ovum pick up in IVF/M vs IVF. Only in six IVF/M patients (10%) a

Table 1
Basal characteristics of patients enrolled.

	IVM/F	IVF	Two tailed p-value
Age (years)	27.9 ± 3.6 Range (19–40)	32.07 ± 4.09 Range (20–43)	0.5609
BMI (Kg/m^2)	27.3 ± 4.6	26.3 ± 5.9	0.2398
Years of infertility	4.5 ± 3.17	5.2 ± 3.7	0.1977
Primary infertility	81.4% (48/59)	81.7% (134/164)	1.0000
ART indications			
Tubal factor	32.2% (19)	29.3% (48)	0.7410
Male factor	28.8% (17)	31.7% (52)	0.7442
IUI failure	25.4% (15)	28% (46)	0.7368
Unexplained	13.5% (8)	11% (18)	0.6378
AFC	19.3 ± 4.6	17.5 ± 9.5	0.1641
FSH (IU/mL)	6.2 ± 1.8	6.6 ± 8.0	0.7042
LH (IU/mL)	8.30 ± 5.1	7.8 ± 3.8	0.4316
E2 (pg/mL)	39.7 ± 13.5	43.7 ± 29.4	0.3153
Testosterone (ng/dL)	0.66 ± 1.2	0.62 ± 1.2	0.8264

mature oocyte was found in IVF/M on the day of retrieval. If so, it was decumulated and injected by ICSI the same day of the recovery, embryos from mature oocytes were transferred with the other embryos resulted from IVM for a mean number of embryos 2.8 ± 0.8 vs 2.43 ± 0.6 in IVF (p = 0.3700) (Table 2). Findings on embryo quality, embryos transferred and results in terms of pregnancy, implantation abortion and delivery rate are described in Table 2. Embryo quality in IVF/M group was lower, with a decreased cleavage rate 84.6% ± 4.9 vs 90.5% ± 5.0 (p = 0.0001) and less good quality embryos 43.3% vs 63.4% in IVF (p = 0.0001) assessed by morphology score. The number of good quality embryos per cycle was 3.1 ± 2.5 in IVF/M vs 6.1 ± 2.3 in IVF group (p = 0.0001).

Only two patients in IVM/M group were not transferred (Table 2) because of deficient endometrial growth, but they generated transferable good quality embryos which were vitrified. As it is expected, endometrial thickness is significantly decreased in IVF/M group (Table 2). So far, no pregnancies have been obtained with frozen-thawed embryo transfer on IVF/M group. In the fresh embryo transfer, pregnancy rates per cycle were 39% vs 53.6% (p = 0.0682). Delivery rate per cycle was 30.5% vs 44.8% (p = 0.1209) in IVF/M vs IVF. 23 pregnancies from IVF/M cycles (6 doubles and 17 singletons) were obtained leading to 5 miscarriages and 18 deliveries of 24 healthy babies. No major or minor malformations were found in both groups. Until now, six frozen embryos transfers have not produced pregnancies. We hypothesize, this is related with the lower quality of embryos in IVF/M group (Table 2).

Discussion

In Mexico and other Latin-American countries, experience with IVM is restricted; an initial attempt in Mexico was reported using only hCG priming [8]. Our center previously reported a case of IVM in a normoovulatory female for treatment of azoospermia in her partner [9]. The limited availability of IVM can be related with hurdles to establish a new ART in private settings in low or medium income countries because the cost of treatment is carried completely by the patients.

In our center, IVF/M is offered for all patients with PCOS requiring IVF when they cannot afford the cost of routine IVF. The cost of conventional IVF is about 4000 dollars and the cost of IVF/M is 2000 dollars. Using IVM/M is an innovative approach because IVM is normally only considerer after multiple IVF failures or OHSS [2–4]. Our medium-term goal is to use IVF/M as first line treatment for PCOS. We always provide counseling about the lower success rates compared to conventional stimulation IVF reported in literature but highlighting the advantages such as decreased risk of OHSS and the important reduction in the cost of treatment.

It is not clear which are the clinical indications that may provide an adequate pregnancy rate for IVF/M or IVM. The most important indications are PCOS patients, especially patients with high risk of OHSS and in cancer patients with an oncofertility indications, such as estrogen sensitive malignant tumors and patients requiring rapid oocyte retrieval before gonadotoxic treatment [2,4]. However, IVM was proposed as a “mild and friendly” ART that can be useful despite its lower rates [4]. Emergency fertility preservation is gaining popularity because cancer treatment in young women is more frequent, this is an important indication to have an active program of IVM [2]. Our clinic is also moving to this approach for fertility preservation when ovary stimulation is not possible.

Our results are comparable to IVF in Latin America; clinical pregnancy rate per cycle is 31% for ICSI cycles according 2014 report of Latin-American registry of assisted reproduction [10]. Therefore, our results of 39% clinical pregnancy rate with IVF/M are similar to the overall clinical pregnancy rate of ICSI in Latin America. Up to now, IVM is considered as an experimental procedure according the ASRM [2], because the best available evidence indicates lower pregnancy rates. This fact limits its application in US and other Latin American countries.

In the other hand, the latest ESHRE report of assisted reproduction in Europe describing the cycles practiced in 2013 indicates that ten countries are performing IVM, mainly in France (48% of the cycles) and Poland. In total, there were 247 cycles that represents a decrease compared to previous years (421 in 2012, 511 in 2011); IVM corresponds only to 0.3% of the 688,271 cycles performed in Europe in 2013 [11]. From the 247 aspirations recorded, 137 transfers were performed resulting in 35 pregnancies and 25 deliveries. (25.5% pregnancy rate and 18% delivery rate per transfer). Our pregnancy rates and delivery rates are higher than Europe. This can be related with the selection of patients, differences in drugs used or the good prognosis of patients recruited in our cohort.

Table 2
Clinical, laboratory and obstetrical outcomes of IVF/M cycles vs conventional IVF.

	IVF/M	IVF	Two tailed p-value
Number of cycles	59	164	
Number of cycles with ET (%)	98.3% (57)	92.3% (156)	1.0000
Patients with OHSS	0% (0/59)	6.7% (11/164)	0.0399
OCC recovered	16.9 ± 9.4	18.0 ± 9.8	0.4557
Cycles with MII oocyte at retrieval	10.1% (6)	NA	
Number of MII oocytes	At 24 h	At decumulation	0.0006
	9.4 ± 4.5	12.4 ± 6.0	
Maturation rate at 24 h (%)	63.2 ± 6.8	NA	
Fertilization rate (%)	87.7 ± 4.68	86.7% ± 5.67	0.2262
Cleavage rate (%)	84.6 ± 4.9	90.5% ± 5.0	0.0001
Percentage of good quality embryos	43.3% (183/422)	63.4% (1002/1580)	0.0001
Number of good quality embryos per cycle	3.1 ± 2.5 (183)	6.1 ± 2.3 (1002)	0.0001
Number of transferred embryos	2.8 ± 0.8 (160)	2.43 ± 0.6 (381)	0.3700
Endometrial thickness (mm)	7.6 ± 1.9	8.3 ± 2.0	0.0204
Clinical pregnancy rate/cycle	39.0% (23/59)	53.6% (88/164)	0.0682
Clinical pregnancy rate/ET	40.3% (23/57)	56.4% (88/156)	0.0444
Implantation rate	18.1% (29/160)	31.2% (119/381)	0.0021
Abortion rate	21.7% (5/23)	20.4% (18/88)	0.5590
Multiple pregnancy rate	26.1% (6/23)	29.5% (26/88)	0.8026
Delivery rate per cycle	30.5% (18/59)	42.6% (70/164)	0.1209
Delivery rate per transfer	31.6% (18/57)	44.8% (70/156)	0.0864
Newborns	24	96	
Gestational weeks	36.2 ± 2.2	37.1 ± 2.3	0.864
Weight	2620 ± 470 g	2826 ± 652 g	0.1486

Changes in terminology for IVM have been recently proposed based in the type of hormonal priming [12]. While in the past IVM was considered even if FSH or hCG priming were given, nowadays it is considered as natural IVF/M when hormonal priming is used. This will affect how IVM data are collected in the future. Controversy exists in relation to a negative effect of hCG and a Cochrane review proposed a harmful effect [13]. More research is urgently needed to optimize the protocols and standardize terminology [4].

An interesting finding in this study was the relative high (26.1%) multiple pregnancy rate, no significantly different ($p = 0.8026$) from the 29.5% of conventional IVF. This is a clear reminder that despite IVM offers protection from OHSS in PCOS patients (Table 2), IVM is associated to an important risk of multiple pregnancies. This risk should be appropriately counseled and potentially avoided by transferring less embryos or moving to single embryo transfer. Elective single blastocyst transfer in IVM cycles with similar results of day 3 transfer has been reported [14]. Further research of IVM in good prognosis PCOS patients, like our cohort, is needed to clarify the optimal number of embryos to transfer and if blastocyst culture can be combined with IVM to effectively increase delivery rates.

Despite the limitations of small sample size, lack of randomization and patients coming from a single center, our results suggest that IVF/M can be used in patients with PCOS requiring IVF with satisfactory delivery rates. IVF/M provides a budget friendly approach for patients covering out of the pocket the cost of infertility treatment. Further randomized studies are required to optimize the best IVM protocols, along with a detailed health economics study comparing IVF/M and IVF in PCOS patients.

Conflict of interest

The authors have no conflicts of interest relevant to this article.

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References

[1] Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril 1994;62:353–62.
[2] Practice committees of the American society for reproductive medicine and the society for assisted reproductive technology. In vitro maturation: a committee opinion. Fertil Steril 2013;99(3):663–6.
[3] Nogueira D, Sadeu JC, Montagut J. In vitro oocyte maturation: current status. Semin Reprod Med 2012;30:199–213.
[4] Chian RC, Uzelac PS, Nargund G. In vitro maturation of human immature oocytes for fertility preservation. Fertil Steril 2013;99:1173–81.
[5] Chian RC, Xu CL, Huang JY, Ata B. Obstetric outcomes and congenital abnormalities in infants conceived with oocytes matured in vitro. Facts Views Vis Obgyn 2014;6:15–8.
[6] Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2014; 19:41–7.
[7] Son WY, Yoon SH, Lim JH. Effect of gonadotropin priming on in-vitro maturation of oocytes collected from women at risk of OHSS. Reprod Biomed Online 2006;13:340–8.
[8] Dávila-Garza SA, Santos-Haliscak R, García-Villafaña G, Lara-Rangel EL, Sepúlveda González J, Díaz-Spíndola P. Ciclo natural combinado con maduración in vitro de ovocitos: la experiencia inicial en México. Rev Mex Reprod 2011;4:82–5.
[9] González-Ortega C, Piña-Aguilar RE, Cancino-Villareal P, Gutiérrez-Gutiérrez AM. Birth after human chorionic gonadotropin-primed oocyte in vitro maturation and fertilization with testicular sperm in a normo-ovulatory patient. J Hum Reprod Sci 2016;9(3):210–2.
[10] Zegers-Hochschild F, Schwarze JE, Crosby J, Musri C, Urbina MT. Latin American network of assisted reproduction (REDLARA). Assisted reproduction techniques in Latin America: the Latin American registry, 2014. Reprod Biomed Online 2017;21. pii: S1472-6483(17)30259-6.
[11] European IVF-monitoring Consortium (EIM); European society of human reproduction and embryology (ESHRE), Calhaz-Jorge C, De Geyter C, Kupka MS, de Mouzon J, Erb K. Assisted reproductive technology in Europe, 2013: results generated from European registers by ESHRE. Hum Reprod 2017;32:1957–73.
[12] Dahan MH, Tan SL, Chung J, Son WY. Clinical definition paper on in vitro maturation of human oocytes. Hum Reprod 2016;31:1383–6.
[13] Reavey J, Vincent K, Child T, Granne IE. Human chorionic gonadotrophin priming for fertility treatment with in vitro maturation. Cochrane Database Syst Rev 2016;16(11), CD008720.
[14] Hatırnaz S, Hatırnaz E, Dahan MH, Tan SL, Ozer A, Kanat-Pektas M, et al. Is elective single-embryo transfer a viable treatment policy in in vitro maturation cycles? Fertil Steril 2016;106:1691–5.