In vitro Maturation of Oocytes in High Altitude Women with Polycystic Ovaries

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ABSTRACT

Objective: Determine the effectiveness of in vitro maturation of oocytes in the infertility treatment in high altitude women with polycystic ovaries.

Methods: Design: descriptive and retrospective study. Participants: Women with polycystic ovaries and infertility. Interventions: there were 11 women from locations above 7,546 feet above sea level with polycystic ovaries and infertility in which were performed in vitro maturation of oocytes, followed by intracytoplasmic sperm injection, culture and embryo vitrification. After that, the endometrium was prepared and the embryos were thawed and transferred. Main results measurements: oocytes maturation, fertilization, clinical pregnancy and implantation rates.

Results: Oocytes maturation rate was 86.1%; fertilization rate 90.3%; clinical pregnancy rate 36.4% and implantation rate 17.4%.

Conclusions: In vitro maturation of oocytes is an effective technique in the infertility treatment of high altitude women with polycystic ovaries.

Keywords: In vitro maturation of oocytes, IVM, Polycystic ovaries, PCOS, IVF, ICSI.

INTRODUCTION

Polycystic ovaries (PO) and the polycystic ovary syndrome (PCOS) are frequent conditions in general population, such as in the group of patients who are treated for infertility. PCOS was first described in 1935 by Irvine Stein and Michel Leventhal through a report of seven patients: four of them were obese, with amenorrhea and big ovaries with polycystic appearance. They performed a wedge resection in the seven patients, achieving ovulation in all of them and pregnancy in two (Pacheco, 2008).

While the term "polycystic ovary" is referred to the aspect of the ovary in the ultrasound, the PCOS is diagnosed when this aspect in the ultrasound is associated to symptoms and signs of hyperandrogenism or ovarian dysfunction (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004; Pacheco, 2008). In Peru, Noriega (1989) finds PO in 28.8% of the 104 volunteer women. It even reports PCOS characteristics in the group, such as menstrual irregularities in 50%, overweight in 17% and hirsutism in the 76%. Inside an infertility patient group, Gasco (1992) reports that 22.4% of the causes are by an endocrine ovarian factor and inside of it, 31.9% has chronic anovulation and 18.8% has PCOS. In Centro de Fertilidad y Ginecología del Sur (CFGS), 18.9% of the patients who require in vitro fertilization (IVF) have PO.

In women with PO, the stimulation with gonadotropins, necessary for IVF procedures, can generate excessive response with ovarian hyperstimulation syndrome with hemodynamic instability and life risk for the patient. It can also generate a poor response with few mature oocytes and poor quality. On the other side, those patients with

appropriate ovarian response, may have low implantation rates due to high estrogen level and deleterious effects in the endometrial receptivity. Against these risks that produces ovarian stimulation in PO patients, in vitro maturation (IVM) of oocytes is constituted as an alternative technique.

Peru has a big geographic diversity, with populated centers, both at sea level and high altitudes. Around 30% of peruvian population lives above 9,842 feet above sea level (F.A.S.L). Menarche takes longer, and menopause happens earlier, because of a high altitude factor and not because of racial, nutritional, or socioeconomic differences, shortening the reproductive life of the andean woman (Reynafarje, 1990). The pathologies related to infertility are probably similar at sea level and at high altitude. However, earliest decrease of the ovarian reserve is an additional factor that motivates the realization of IVF procedures at high altitude.

IVM of oocytes consists in obtaining immature oocytes (in germinal vesicle state) from antral ovarian follicles without or minimally stimulated with gonadotropins before being matured under laboratory conditions in culture supplemented with hormones. This technique allows us to obtain oocytes in metaphase II in order to be able to be fertilized without needing elevated gonadotropin doses.

IVM of oocytes was proposed by Pincus and Enzmann in 1935 (Pincus & Enzmann, 1935) and then by Edwards, in 1969 (Edwards *et al.*, 1969). In 1991, the first pregnancy product of IVM of oocytes was achieved (Cha *et al.*, 1991), and now there are more than 2,000 children achieved through this technique. The first peruvian child product of IVM of oocytes was born in 2012, whose mother required such technique because of anovulation associated with amenorrhea and anorexia (Vargas *et al.*, 2012a; Vargas *et al.*, 2012b).

The present study seeks to determine the effectiveness of IVM of oocytes in the infertility treatment in high altitude women with PO.

MATERIAL AND METHOD

It is a retrospective and descriptive study of 11 women with PO and infertility diagnosis in whom IVM was carried out, from June 2011 to October 2013. The procedures were done in the Embryology Laboratory of the CFGS, in the city of Cusco, at 10,925 F.A.S.L. Patients were from regions of Cusco, Apurimac and Puno (locations above 7,546 F.A.S.L).

It is defined as PO to a number of antral follicles (AFC) of 12 or more (follicles between 2 and 9mm) in one or both ovaries (Pacheco, 2008; Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). The characteristics of the patients are described in Table 1.

75 to 150 IU of rFSH for 3 consecutive days were administered between the first and fifth day of the cycle. Human chorionic gonadotropin (hCG) was not administered. Between the sixth and eighth day of the cycle follicular as-

Table 1. Characteristics of the patients		
Characteristics		Media
Age	27-39 years old	33.6
AFC	19-36	22.4
Diagnosis	Anovulation (4), Male factor(4), Unexplained infertility (2), Endometriosis (1)	

*AFC: Antral follicle count

piration under intravenous sedation was carried out using an 18 gauge needle of single lumen (Vitrolife) or immature oocytes aspiration needle (Cook).

The cumulus-oocytes complex (COC) (Fig. 1A) were cultivated in maturation medium with 75 mIU/ml of rFSH (Gonal-F, Merck-Serono) and 100 mIU/ml of hCG (Pregnyl, Schering-Plough or Choragon, Ferring), supplemented with 10% of inactivated patient's serum. After 36 hours, the COC were evaluated (Fig. 1B) and metaphase II oocytes

were inseminated by intracytoplasmic sperm injection (ICSI) (Fig. 1C) and cultivated in Global medium (LifeGlobal). 18 hours after ICSI was carried out, fertilization of the oocytes was evaluated (Fig. 1D), and these were kept in the same medium for the next 24 hours (Fig. 1E).

Since there is a mismatch between embryonic and endometrial development, embryo freezing by vitrification was performed (Kuwayama *et al.*, 2005) using McGill Cryoleaf™ (Origio) device and were stored in liquid nitrogen.

After 2 to 7 months, at the beginning of menstruation, estradiol valerate (Progynova) was used in ascendent progressive doses orally from 2 mg to 12 mg daily until having an endometrial thickness above 8 mm. 200 mg of micronized progesterone (Utrogestan, Ferring or Geslutin PNM, Tecnofarma) and 90 mg of progesterone gel (Crinone 8%, Merk-Serono) were added daily, both intravaginal, or the association of 600 mg of micronized progesterone plus the application of the progesterone vaginal ring (Fertiring, ABL Pharma).

Embryos were thawed and cultured for 24 hours (Fig. 1F). Afterwards, they were transferred to the uterine cavity using a Frydman Ultrasoft catheter (CCD Laboratories)

Figure 1. Sequence of the process of IVM of oocytes (A) Immature COC. (B) COC at 36 hours of culture with rFSH + hCG + patient's serum. (C) Denuded oocyte showing the second polar body (metaphase II). (D) Oocyte fertilization and evaluation at 16 hours. (E) Embryo after 42 hours of development and freezed by vitrification. (F) Thawed embryo, cultivated for 24 hours and transferred.

Table 2. Oocyte and embryo development

Results	
Aspirated oocytes	36 (1 to 6 oocytes, 3.3 in average)
Oocytes that accomplish maturation	31 (maturation rate: 31/36 = 86.1%)
Oocytes that accomplish fertilization	28 (fertilization rate: 28/31 = 90.3%)
Oocytes that accomplish cleavage	25 (cleavage rate: 25/28 = 89.3%)
Transferred embryos	22 (1 to 3 embryos, 2.1 in average)
Top quality embryos	12 (12/23 = 52.2%)
Positive β -hCG rate	7/11 = 63.6%
Clinical pregnancy rate	4/11 = 36.4%
Implantation rate	4/22 = 17.4%

under abdominal ultrasound guide.

After 13 days of the transfer, dosage of β -hCG was performed. In case of having a positive result, a vaginal ultrasound was performed 1 or 2 weeks after the test. It is defined as a clinical pregnancy the presence of a gestational sac and presence of an embryo with cardiac activity.

RESULTS

Table 2 describes oocyte and embryo development.

A patient achieved a fully 38 week pregnancy with the birth of a healthy 3,250gr girl through elective caesarean section. A second patient required an emergency caesarean section at the 36 week due to pre-eclampsia, having a healthy 2,390gr girl.

Two patients having ongoing active pregnancy, developed the disappearance of the cardiac embryonic activity in the 8 week. A third patient developed gestational sac without embryo and was considered as a biochemical pregnancy.

DISCUSSION

In our laboratory, IVM of oocytes achieved high maturation, fertilization and cleavage rates (86.1%, 90.3% y 89.3%, respectively). International reports show oocyte maturation rates between 33 and 82%, fertilization rates between 44 and 80% and cleavage rates between 19 and 96% (Farsi *et al.* 2013). Despite the limited number of oocytes obtained by aspiration (3.3 in average), clinical pregnancy rate of 36.4% was achieved, which is between the 17 to 41% rate that is reported for this technique. Even though there are reports that claim an advantage in the administration of hCG before obtaining the oocytes (Son *et al.*, 2008a, 2008b, Tan & Child, 2002, Chian, 2004) it's being also manifested that hCG interrupts communication between follicular cells and the oocyte; this is an important event to the adquisition of a good embryonic potential development (Albertini *et al.*, 2001). In our IVM protocol, we do not use hCG in vivo. We mature the oocyte in vitro putting them in the presence of FSH and hCG in the medium and satisfactory results with this technique were found (De Vos *et al.*, 2011). The advantage of combining IVM of oocytes and vitrification of the embryos, is to postpone embryo transfer when the endometrium is sufficiently receptive.

Abortion development in 2 patients with clinical pregnancy, might be associated to inherent factors, such as polycystic ovaries and age (37 and 38 years old respectively). In CFGS, population that requires assisted reproduction techniques presents a notorious decrease in pregnancy rates from 35 years old (Vargas *et al.*, 2013).

IVM of oocytes provides and effective alternative for high altitude women with PO, obtaining good maturation,

fertilization and cleavage rates without using high dose of ovulation inductors. We consider that to improve clinical strategies to achieve more number of collected oocytes is required, and these will allow us to improve clinical pregnancy rates.

CONCLUSIONS

IVM of oocytes is an effective technique in infertility treatments in high altitude women with PO.

CONFLICT OF INTERESTS

No conflict of interest have been declared.

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