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IN-VITRO ACTIVATION OF OVARIAN FOLLICULAR RESIDUAL RESERVE

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CONDENSATION

The newly developed in-vitro activation (IVA) method provides a novel fertility treatment for patients with premature ovarian insufficiency. The IVA method pretends to promote growth of residual ovarian follicles at early stages of their development. Based on preliminary data, poor ovarian response (POR) patients with decreased ovarian reserve (DOR) who have multiple secondary follicles, IVA is a promising technique to promote growth of secondary follicle as well.

Key words: *premature ovarian insufficiency, in-vitro activation (IVA) method, poor ovarian response (POR)*

INTRODUCTION

Throughout the female life span under physiological circumstances, the majority of ovarian follicles do not enter the stage of cyclic recruitment and undergo atresia until near complete follicular exhaustion coincides with menopause. Approximately 250,000 primordial follicles are present at menarche, whereas only a few hundreds or thousands remain at the end of reproductive life [Block E. 1952].

Age-related decreased fertility has become an increasing challenge. The average age of first-time motherhood has increased dramatically over last decades. Natural fecundity as well as the success of any intervention, including artificial reproductive technology (ART), decreases dramatically with increasing maternal age due to exhaustion of the resting ovarian primordial follicles pool [Broekmans et al., 2009].

Approximately 1% of women suffer from premature ovarian insufficiency characterized by a loss of ovarian activity before 40 years of age. Women suffer from premature ovarian insufficiency (POI), diminishing ovarian reserve (DOR) and poor responders (POR) suffer from ovarian infertility and egg donation has been the only option for having their own child [Huhtaniemi et al., 2018].

A new treatment to improve fertility potential in POI, DOR and POR patients

Histological ovarian samples reveal that the follicle pool in the ovary is not completely exhausted until early in the eighth decade of life and almost 90% of primordial follicles are embedded in the ovary cortex [Gougeon et al., 1994]. The important question raised from this data is how to activate the remaining ovarian "gold reserve" in women with premature cessation of ovarian function and in those who wish to become pregnant at a more advanced age without resorting to egg donation. In the last decade new in vitro and in vivo techniques to solve this issue appeared such as the development of artificial gametes from ovarian cortex in-vitro [McLaughlin et al., 2018], mobilization of stem cells to peripheral blood by granulocyte colony-stimulating factor (G-CSF) and sub-

sequent collection by apheresis followed by autologous stem cell ovarian transplantation (ASCOT) [Herraiz et al., 2018]. Intra-ovarian injection of calcium gluconate-activated autologous platelet-rich plasma (aPRP) was also suggested to address the ovarian response [Sills et al., 2018]. PRP includes cell-activating factors such as vascular endothelial growth factor (VEGF), transforming growth factor (TGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) [Lee et al., 2013]. None of these approaches have entered routine clinical practice, and the evidence available so far is either still pre-clinical or of questionable validity.

In vitro activation (IVA) of the mammalian dormant follicles was reported for a first time in a mice ovary model [Li et al., 2010] and was based on the PTEN-PI3K-Akt-Foxo3 pathway. This approach was adopted as a treatment modality in women diagnosed diminished ovarian reserve by Dr. Kazuhiro Kawamura's group, combining the partial oophorectomy followed by two days in-vitro active stimulation of the ovarian cortex fragments [Kawamura et al., 2013]. The original IVA protocol has later been modified into a drug-free, one-step procedure with laparoscopic retrieval of ovarian fragments, removal of ovarian medulla, dissection of ovarian cortex into small cubes of about 1 mm³ and immediate autologous re-transplantation [Kawamura et al., 2020]. Ovarian tissue fragmentation, or incision, converts globular (G)-actin into filamentous (F)-actin, which inhibits Hippo signaling pathway [Hsueh et al., 2015]. The Hippo signaling pathway, also known as the Salvador-Warts-Hippo (SWH), is involved in cell growth negative regulation. It is an evolutionarily conserved pathway that controls organ size by regulating cell proliferation, apoptosis, and stem cell self-renewal [Cheng et al., 2015]. Hippo pathway gene mutations increased organs such as liver and heart in mice. Hippo signaling disruption alone can promote in turn, stimulation of cell growth, survival, and proliferation that is leading to growth of dormant primordial follicles. Follicles in proximity to cutting line of the tissue develop better than follicles which are imbedded deeper in the cortex tissue [Grosbois and Demeestere, 2018].

A drug-free activation of follicular growth only by mechanical disruption is now being tested in our POI patients. Patient selection is based on primarily on ovarian function and on factors as patient's age. The patient's ovarian dysfunction etiology is variable and includes genetic (FMR1 premutation, chromosomal abnormalities and autosomal aberrations), iatrogenic, infectious and autoimmune disorders.

After the cutting of an ovarian cortex, an auto-transplantation grafting into artificial pouches created beneath the serosa of fallopian tubes [Zhai et al., 2016] is conducted. This IVA procedure promoted follicle growth and allowed the generation of mature oocytes for POI patients.

PATIENT SELECTION FOR IVA

POR patients were recruited with DOR based on the Bologna criteria [Ferraretti et al., 2011], showing the growth of few antral follicles following FSH treatment or with low ovarian reserve who failed to achieve pregnancy following IVF cycles. Their serum gonadotropin, estradiol and serum anti-Müllerian hormone (AMH) levels were monitored. Informed consent was obtained from patients and the study is carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

PATIENT PRE-TREATMENT

Before IVA, patients receive hormone replacement therapy to maintain serum estradiol between 40 and 90 pg/ml, in order to suppress circulating LH and FSH levels. Serum LH and FSH concentrations are monitored to make sure that LH concentrations are less than 10 mIU/ml prior to surgery.

ONE-STEP DRUG-FREE IVA

The IVA procedure involves laparoscopic removal of 1 cm² of the cortex that is plunged into the buffered medium for further preparation of ovarian tissue strips. All cutting procedures are performed by scalpel in the same operation room where the IVA is conducted. For Hippo signaling disruption, ovarian cortex is dissected to remove residual medulla tissues before cutting into strips (approximately 10 mm in length and 1-2 mm width). After dissection, cortex strips are fragmented into small cubes of 1-2 mm³. Depending on the availability of cubes and surgery progress, some cubes are grafted back into the ovaries, pockets in the broad ligament and beneath the serosa of fallopian tubes bilaterally, similar to the original IVA approach [Kawamura et al., 2013]. The grafting back of the multiple cortical cubes (approximately 8-12 pieces/portion) is carried out by using dedicated catheters.

POST-GRAFTING HORMONAL TREATMENTS

After surgery, patients receive estrogen and progesterone for 10-14 days to initiate withdrawal bleeding. Once adequate serum LH levels (<10 mIU/ml) are confirmed, nasal spray of a GnRH agonist (Nafarelin, Synarel) is applied to maintain serum LH concentrations, along with daily injections of FSH (225-300 IU) to maintain elevated serum FSH (>25-30 mIU/ml). Serum LH concentrations are maintained at 1-9 mIU/ml by adjusting the daily GnRH agonists dosage. Serum estradiol, FSH and LH, as well as ultrasound monitoring for all patients, are used as the monitoring modalities.

PATIENT PREPARATION FOR IVF

During monitoring of follicles under transvaginal ultrasound and serum concentrations of hormones following ovarian stimulation, some patients showed initiation of growing follicles at the beginning of ovarian stimulation. The first indication of follicle growth was elevation of serum estradiol and small antral follicles, which became detectable on ultrasound scan when serum estradiol levels reached >100 pg/ml. When growing follicles reached 14-18 mm in diameter, patients are injected with 10,000-20,000 IU hCG to induce the final oocyte maturation before oocyte retrieval. Compared to routine IVF stimulation protocols, higher hCG doses are occasionally used in IVA cycles due to expected poor vasculature of grafts.

IVF in patients after IVA

After oocyte retrieval routine IVF was performed. Culturing embryos to blastocyst stage to select high-quality embryos is not recommended for POR patients, because only limited numbers of embryos could be obtained and prolonged culture does not improve embryo quality in-vitro. Embryo transfer is performed on day 2 or day 3 of embryo fresh cycle or frozen/thaw cycle after endometrium preparation with estrogen followed by progesterone treatment. After embryo transfer, patients received luteal support using vaginal progesterone. Pregnancy is determined by measuring serum β -hCG levels 2 weeks after embryo transfer.

Between January 2020 and October 2020 we perform 12 procedures of IVA. Half of our patients responded to the treatment with improvement in follicle growth (>12 mm) following ovarian hyperstimulation. In these patients we could retrieve more oocytes as compared with number of oocytes per cycle before IVA. One pregnancy after ovarian stimulation and timed intercourse in the period of the first SARS-CoV-2 wave was achieved. At present, other patients are undergoing embryo transfer procedures of fresh and frozen embryos along with continued recruitment of new patients.

DISCUSSION

The present preliminary data suggest that ovarian follicles of POR patients may be activated following drug-free IVA involving cortical fragmentation and auto-transplantation. During one step drug-free IVA procedure, ovarian cubes are grafted immediately after cutting, thus avoiding potential follicle loss during prolonged culture. As orthotopic grafting is performed, some patients could potentially become pregnant naturally without egg retrieval and embryo transfer.

Most POR patients with DOR who underwent drug-free IVA showed follicle growth within several weeks, suggesting the presence of residual secondary follicles and their growth into antral follicles. Consistent with previous findings in POI patients indicating serum AMH levels are not predictive of follicle growth after IVA [Kawamura et al., 2013], and even these patients are also responded to IVA treatment, allowing the retrieval of oocytes.

It is important to note that the IVA approach increases the number of mature oocytes retrieved, but does not improve age-related decline in egg quality, especially increased rates of aneuploidy, in POR patients [Kailasam et al., 2004]. Therefore, the IVA approach is more effective in younger POR patients. As oocyte quality decline is a random process, retrieval

of a large number of mature oocytes after IVA in middle-aged patients may allow the possibility of successful pregnancy.

As our experience and the other published studies involve

a small group of patients, the results are preliminary and should be interpreted with caution.

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ТҮЙІНДЕМЕ**ЖАТЫР Фолликулаларының қалдық қорын экстракорпоралды белсендіру****Е. Гирш, А. Харлев, Л. Грин**

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Жақында эзірленген экстракорпоралды белсендіру әдісі (IVA) аналық безінің жеткіліксіздігі ерте басталған емделуші әйелдерді бедеуліктен емдеудің жаңа әдісін ұсынады. IVA әдісі жатырдың қалдық фолликулаларының дамуының бастапқы кезеңінде олардың өсуіне ықпал етуге арналған. Алдын ала мәліметтерге сүйенсек, аналық безінің қоры төмендеуінен (DOR) аналық безінің жеткіліксіз жауабы (POR), көптеген қайталама фолликулалары бар емделушілер үшін IVA екінші реттік фолликулалардың өсуін ынталандырудың перспективті әдісі болып табылады.

Түйін сөздер: ерте басталған аналық безінің жеткіліксіздігі, экстракорпоралды белсендіру әдісі (IVA), аналық бездердің жеткіліксіз жауабы (POR).

РЕЗЮМЕ**IN-VITRO АКТИВАЦИЯ ОСТАТОЧНОГО РЕЗЕРВА Фолликулов в яичниках****Е. Гирш, А. Харлев, Л. Грин**

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Недавно разработанный метод экстракорпоральной активации (IVA) представляет собой новый способ лечения бесплодия у пациенток с преждевременной овариальной недостаточностью. Метод IVA призван способствовать росту в яичниках остаточных фолликулов на ранней стадии их развития. На основании предварительных данных, для пациенток с недостаточным ответом яичников (POR), со снижением овариального резерва (DOR), но имеющих множественные вторичные фолликулы яичников, IVA также является перспективным способом для стимулирования роста вторичных фолликулов.

Ключевые слова: преждевременная овариальная недостаточность, метод экстракорпоральной активации (IVA), бедный ответ яичников (POR).