Update of In Vitro Activation (IVA) for Infertility Treatments in Patients with Primary Ovarian Insufficiency and Ovarian Dysfunction

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Primary ovarian insufficiency (POI) affects about 1% of women in the reproductive age and show severe infertility due to decreasing ovarian reserve and amenorrhea. With delayed childbearing being the current norm in many countries, infertility is rapidly emerging in POI patients. Although it is difficult to change the delayed childbearing in modern society, recent advances in reproductive medicine allows us to predict POI before onset of complete amenorrhea. In addition to traditional antral follicle count in ovaries under transvaginal ultrasound, measurement of anti-Müllerian hormone is a useful tool to diagnose POI before onset of complete amenorrhea. After prediction of POI, the development of techniques for cryopreservation of oocytes, embryos and ovarian tissue enables us to preserve fertility in POI patients. Although the oocyte donation is promised approach for infertility treatment in POI, there are several concerns to use OD for their infertility treatment.

POI patients are infertile due to a lack of follicle growth and ovulation, because residual ovarian follicles in these patients are not responsive to traditional gonadotropin treatments. To induce follicle growth in these remaining dormant follicles, we have developed a method for activation of dormant follicles by using in vitro culture of ovarian fragments treated with PI3K stimulators following disruption of Hippo signaling pathway (IVA, in vitro activation).

Our and previous studies showed the importance of PI3K-Akt-Foxo3 signaling in activation of dormant primordial follicles. We demonstrated the activation of dormant follicles using a PTEN inhibitor and PI3K activator based on a short term (48 hours) in vitro activation protocol in mice and human ovaries, leading to increased primordial follicle number (PNAS 2010). Furthermore, we generated mature oocytes displaying normal epigenetic regulation in imprinted genes (PNAS 2010). Following the success of these basic and translational studies, we applied the IVA for clinical study to treat infertility in POI patients. In the clinical study, we removed ovaries and fragmented ovarian cortex into small cubes for tissue culture. After the culture, the ovarian cubes were transplanted beneath the serosa of Fallopian tubes following IVF-ET. This approach included histological analyses to detect residual follicles, which allowed predicting the outcome of follicle growth after IVA. We have reported two deliveries of healthy babies after IVA treatment (PNAS 2013 PNAS, Hum Reprod 2015) and the number of babies is increasing. Furthermore, at least seven

other pregnancies by three other centers in Spain, China, and Poland have also been achieved.

In this presentation, I will introduce our IVA procedure and update clinical outcome of IVA so far. Furthermore, I will introduce the Drug-free IVA conducting Hippo signal disruption only as a new infertility treatment for patients with ovarian dysfunction. Although these patients become poor responders due to limited number of antral follicles to respond FSH stimulation, they still have activation of dormant primordial follicles. Because secondary follicles could stimulate to grow by disruption of Hippo signaling, this method allowed to increase the number of antral follicles, leading to increase the number of retrieved oocytes after ovarian stimulation.