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Infertility Treatment in Primary Ovarian Insufficiency: Fertility Preservation and *In Vitro* Activation

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Abstract

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-Mullerian 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 tissues enables us to preserve fertility in POI patients. Although the oocyte donation is an established approach for infertility treatment in POI, patients cannot have their own genetic offspring. Recently, we developed a new infertility treatment, IVA (*in vitro* activation), to promote residual follicle growth and generate mature oocytes, followed by *in vitro* fertilization and embryo transfer in POI patients. Using the IVA treatment, we reported successful deliveries by POI patients and they could have their own genetic children. Currently, the IVA treatment is expanding to more infertility centers world-wide. However, IVA is ineffective in the patients whose follicles are depleted. Thus, it is important to enlighten young women about POI and recommend them to receive early check up of ovarian reserve before the onset of POI.

Keywords: POI; IVA; Cryopreservation; Donor egg

Abbreviations: POI: Primary Ovarian Insufficiency, DOR: Diminishing Ovarian Reserve, IVA: *In Vitro* Fertilization, FSH: Follicle Stimulating Hormone, LH: luteinizing hormone, AMH: Anti-Müllerian Hormone, IVF-ET: *In Vitro* Fertilization and Embryo Transfer, GnRH: Gonadotropin Releasing Hormone, PI3K: phosphoinositide 3-kinase, FOXO3: fork head box O-3

Introduction

Primary ovarian insufficiency (POI) affects about 1% of women in the reproductive age. These women are below 40 years of age, experience amenorrhoea for at least 4 months, and have two readings of serum follicular stimulating hormone (FSH) of more than 40IU/L at one month apart [1-3]. Those who plan to expand their families are mostly left with the option of adoption or oocyte donation. However, donor oocyte is not an option for some of these women due to personal and religious reasons, and is in rare supply. With delayed childbearing being the current norm in Japan and many other countries, where the average age of having the first child has increased to 31 years of age in Japan, sub-fertility is rapidly emerging as a common presentation. Furthermore, this situation is associated with increases in the number of infertile 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, preserve fertility in POI patients, and establish new infertility treatment for POI patients.

Diagnosis of POI before the onset of complete amenorrhea

At birth, female infants have a non-renewable pool of 800,000 quiescent follicles in ovaries. Intraovarian mechanisms activate a small number of dormant primordial follicles to initiate growth [4]. Through activation of dormant follicles and occurrence of follicular atresia, the ovarian reserve decreases with age and follicle depletion occurs at menopause when less than 1,000 follicles remain [5]. The initial symptom of decreasing ovarian reserve is characterized by menstrual irregularities. Because this symptom is quite common in women at reproductive age, irregular menses could not be a good diagnosis for consulting doctors. Although antral follicle count in ovaries under

transvaginal ultrasound is a powerful tool for diagnosis of decreasing ovarian reserve [6], young women likely hesitate to receive gynecological examinations. Traditional gonadotropin examination, namely serum FSH and luteinizing hormone (LH) levels, has been utilized, but it requires blood drawing at days 3-5 of the menstrual cycle due to dramatic changes in serum FSH during menstrual cycle. This limited window of examination makes it difficulties for female students and working women to visit the hospital. Furthermore, serum FSH levels do not correlate the number of residual follicles in ovaries, resulting in inability to predict POI before onset complete amenorrhea.

Anti-Mullerian hormone (AMH) is produced from granulosa cells of primary to small antral follicles and gradually disappear in the large antral and preovulatory follicles [7]. Thus, serum AMH levels reflect the number of primary and secondary follicles. Recent papers demonstrated small changes in serum AMH levels during the menstrual cycle and such changes were not evident in patients with low basal AMH levels [8]. Because patients with low basal AMH levels have few FSH responsive preantral and small antral follicles, their AMH levels unlikely change during the menstrual cycle. Thus, AMH levels still can be determined at any time during menstrual cycle in patients to evaluate ovarian reserve. Because follicles do not regenerate after birth, AMH levels continue to decline with age and pathophysiological conditions. This fact prompts us to diagnose POI before onset of complete amenorrhea. However, some women with undetectable AMH levels still show regular menses. Furthermore, diminution rate of follicles could be different among POI patients due to diverse causes of POI. To overcome these limitations in predicting POI, establishment of biomarkers derived from dormant primordial follicles is needed.

Fertility preservation in POI patients

Because decreasing ovarian follicle reserve during aging and under pathophysiological conditions is progressive and the follicles are not regenerated after birth in nature at least inside of body, cryopreservation of oocytes, embryos and ovarian tissues is an attractive method for fertility preservation in POI patients. Although embryo freezing is an established procedure for in in vitro fertilization and embryo transfer (IVF-ET), recent advances in cryobiology allow us to cryo preserve oocytes and ovarian tissues [9].

For fertility preservation in patients with low ovarian reserve, unmarried patients before onset of complete amenorrhea are good subjects. The birth rate achieved from one cryo preserved oocyte was reported to be 8% in patients with<35 years of age [10]. Due to poor response to gonadotropin stimulation in patients with diminishing ovarian reserve, many cycles of oocyte retrieval after long term gonadotropin treatment will be needed to obtain sufficient number of oocytes for one successful pregnancy.

Although prediction of the number of residual follicles in POI patients needs to be improved, single women could consider oocyte freezing if their AMH levels are <0.1ng/ml, suggesting diminishing ovarian reserve. Indeed, early intervention allows us to prevent decline of oocyte quality during aging. Furthermore, it minimizes medical expenses borne by patients, because higher number of oocytes could be obtained per oocyte retrieval at younger ages for patients with diminishing ovarian reserve for cryopreservation.

Alternatively, patients with low ovarian reserve can cryopreserve ovarian cortical tissues where residual follicles are located [11]. Although limited numbers of pregnancy and delivery are reported after the ovarian tissue freezing [12], this method is suitable for young girls before onset of menstruation even though they are not good candidates for oocyte retrieval. In contrast to oocyte cryopreservation, ovarian tissue freezing requires only one intervention under laparoscopic surgery to the patient, resulting in lower cost as compared to that of oocyte freezing. Although ovarian tissue freezing is a more invasive procedure, it could minimize costs and for patients. Due to increases in single working women in modern society, some young patients might choose to use cryopreserved oocytes or ovarian tissues. Thus, medical expenses and hospitalization time is crucial for these patients. To overcome the disadvantages of ovarian tissue freezing, we can perform oocyte cryopreservation simultaneously by collection of immature oocytes from antral follicles followed by *in vitro* maturation [13]. It is important to note that the data on clinical outcome for pregnancy is still insufficient to determine which method is superior for fertility preservation for patients with lower ovarian reserve.

Infertility Treatment for POI Patients

Oocyte donation

The first pregnancy achieved using donated eggs was reported in 1984 [14]. After initial success, the number of oocyte donation (OD) cycles has increased dramatically world-wide. In combination with blastocyst transfer and pre implantation genetic diagnosis/screening, the success rate of pregnancy using OD from young women is very high [15,16]. Although this high success rate is attractive for POI patients to bear children, there are several concerns to use OD for their infertility treatment. One of the major concerns is the fact that patients cannot have their own genetic children, leading to personal and ethical issue. In some countries, OD is prohibited due to ethical issue (e.g. Japan) and religious reasons (e.g many Islamic countries). Furthermore, some papers reported that OD resulted in high risk pregnancies due to immune compatibility [17] and should be managed carefully by an appropriate obstetric team [18,19]. Because the costs of OD are usually high as compared to regular IVF treatment, profit motives complicate this procedure. Despite these issues, OD is still the last hope for having children for in POI patients.

Ovarian stimulation

Due to few numbers of residual preantral and antral follicles in POI ovaries, it is extremely difficult to obtain follicle growth

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even under ovarian hyper-stimulation using gonadotropins [20]. In POI patients, their low levels of estrogen induce chronic high levels of FSH due to a lack of negative feedback to hypothalamus-pituitary-axis by estrogens. It has been believed that such elevated FSH induces down-regulation of FSH receptors in the follicles, resulting in decline of ovarian responsiveness to FSH stimulation [21]. Although aberrant FSH receptor was not confirmed by in vitro and animal studies, ovarian stimulation has been conducted by correcting high levels of FSH to allow restoration of FSH receptors following administration of exogenous estradiol. To suppress elevated serum of FSH in POI patients, gonadotropin releasing hormone (GnRH) agonist or antagonist treatment have also been is also useful. Together with exogenous FSH to stimulate follicles growth, GnRH agonist treatment could improve follicle growth in POI patients [22]. The advantage of this method is the prevention of early LH surge leading to early luteinization [23]. However, these methods are effective only in cases when patients have spontaneous growth of primordial follicles, thus allowing further follicle growth after FSH stimulation.

IVA

Because of inability to predict the spontaneous activation of primordial follicles and follicle development onward, POI patients need to endure continuous ovarian stimulation to obtain her own oocytes. Because there is no established method to diagnose the exact numbers of residual follicles, some patients may receive ovarian stimulation for a prolonged duration even though they have no residual follicles. To overcome these issues, we developed the method for artificial activation of dormant primordial follicles using in vitro culture of ovarian cortical tissues (IVA: in vitro activation) [24]. Our and previous studies showed the importance of PI3K-Akt-Foxo3 signaling in the activation of dormant primordial follicles [4,25,26]. We demonstrated the activation of dormant follicles using a PTEN inhibitor and a PI3K activator based on a short term (48 hours) in vitro activation protocol in mice and human ovaries, leading to increased primordial follicle numbers [4]. Furthermore, we generated mature oocytes displaying normal epigenetic regulation of in imprinted genes [4]. Following the success of these basic and translational studies, we applied the IVA method 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 followed by IVF-ET. This approach also included histological analyses of neighboring tissues to detect residual follicles, which allowed for predicting the outcome of follicle growth after IVA.

We have reported two deliveries of healthy babies after IVA treatment [24-27] and the number of babies is increasing. After our publication of IVA, two separate centers repeated the IVA procedure [28] and one successful delivery was reported [29]. The IVA for POI patients is currently expanding to more infertility centers in the world.

Conclusion

Recent progress of reproductive medicine allows predicting POI before onset and cryopreservation of oocytes, embryos and ovarian tissue is available to preserve fertility in POI patients. OD is the most successful treatment option for POI patients' infertility treatment, but it is not accepted in all patients due to diverse personal, societal and religious issues. In addition to ovarian stimulation, we developed the new IVA approach for POI patients to obtain their own genetic oocytes. However, its success depends on the number of residual follicles as well as patient age. Taken together, from a view point of women's health, it is very important to enlighten young women about potential for POI and recommend them to receive check ups of ovarian reserve as early as possible when they experience irregular menses and amenorrhea.

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