Specific features

- Lack of follicle growth and ovulation
- Exhaustion of ovarian follicles and few residual follicles (undetectable AMH levels)

Treatments

- Resistant to traditional gonadotropin treatments
- Egg donation is the most successful treatment option, but…
Life History of Ovarian Follicles

INITIAL RECRUITMENT
- Primordial follicle
- Primary follicle
- Secondary follicle
- Antral follicle
- Atresia

CYCLIC RECRUITMENT
- Maturation
- Atresic

Exhaustion of Follicles

Diverse local factors contribute to activation of dormant primordial follicles.

Which intracellular signaling system is involved in the activation?

Growth factors (kit ligand, neurotrophins, BMPs, VEGF, LIF, etc.)
At early stage after birth, PTEN or FOXO3 deletion led to the activation of dormant primordial follicles and resulted in depletion of follicles within 16-18 weeks.
The PI3K signaling pathway begins PI3K activation by receptor tyrosine kinases (RTKs) after binding growth factors. PI3K activates AKT, which inhibits the activities of FOXO3, resulting in cell proliferation and survival. PTEN negatively regulates PI3K signaling.

In primordial follicles, local factors activate dormant follicles through PI3K-Akt-Foxo3 signaling pathway, whereas PTEN acts to block the signaling.
Is it possible to activate residual dormant follicles in POI patients artificially by transient PTEN suppression and/or PI3K activation?
**PTEN inhibitor**

A vanadyl complexed to hydroxypicolinic acid is a highly potent and specific inhibitor at nano-molar concentrations.

**PI3K activator**

A cell-permeable phospho-peptide (740Y-P) binds to the SH2 domain of p85 regulatory subunit of PI3K and activates enzyme activity.

Derossi et al. BBRC, 1998
Xeno-transplantation of human ovarian fragments to activate dormant follicles: IVA, in vitro activation

Ovarian cortical fragments were obtained from patients with benign ovarian tumor with informed consent from the patient and approval from local ethical human subject committee.

Human ovarian cortical fragments

- PTEN Inhibitor
- Control

Xeno-transplanted into kidney capsule

Ovariectomized SCID mice

FSH treatment for 6 months

hCG treatment

Oocyte retrieval

Mature oocytes
Morphology of human ovarian fragments after 6 months of xeno-transplantation
Histology of PTEN inhibitor treated ovarian fragments

At 36 h after hCG treatment, large antral follicles in the PTEN inhibitor-treated group contained mature oocytes at metaphase II accompanied with cumulus expansion.
Clinical application of IVA for POI patients

- Ovariectomy under laparoscopic surgery
- Preparation of ovarian cortical strips for freezing
- Fragmentation of ovarian strips to cubes
- Culture of ovarian cubes with PI3K activators
- Auto-transplantation of activated ovarian cubes
- Retrieve mature eggs
- In vitro fertilization
- embryo cryopreservation
- embryo transfer
- Histological analyses
Enrolled patients

83 of POI patients (37.4 ± 4.9 years of age)

Duration of amenorrhea: 5.7 ± 3.5 years
- Ovariectomy under laparoscopic surgery
- Minimum usage of electrocautery hemostasis to avoid damage of residual follicles.
- Dissect ovarian cortices containing residual follicles by removing medulla.

- Cut into small strips (1 x 1 cm², 1-2 mm thickness).

- Using 10% of volume of each ovarian stripe, detect residual follicles by histological analyses.

- Cryo-preserve by vitrification method.
Ovarian cortical tissues for vitrification

Before dissection of medulla

After dissection of medulla

Small ovarian stripes ready for vitrification (6-8 stripes/POI ovary)

CryoSupport

Composed of four stainless needles inserted into the cap of a cryogenic vial

vitrification

Croprotectant A

Croprotectant B

Croprotectant C

Liquid N2

After successful preparation, cortical stripe appeared transparent
- Thaw some (2-3 pieces) ovarian stripes
- Fragment into 1-2 mm$^2$ of cubes
- IVA drugs treatment (PTEN inhibitor and PI3K activator) for 2 days to activate dormant follicles
• Before auto-transplantation, wash cultured ovarian cubes by warmed culture media alone.

• Transplant beneath the serosa of Fallopian tubes (20-40 cubes per site).

Beneath serosa of Fallopian tubes — high vascularization, convenience for trans-vaginal ultrasound monitoring ease for oocyte retrieval
Auto-transplantation

Cutting the serosa and making a pouch between serosa (arrows) and Fallopian tube (arrowhead).

Grafting multiple ovarian cubes (arrows) beneath the serosa of Fallopian tubes.

Wound was covered by an oxidized regeneration cellulose to avoid cube loss from the graft site.
Movie for auto-transplantation
Monitor follicle growth weekly to biweekly: transvaginal ultrasound + serum estrogen and gonadotropin levels.

When estrogen levels were increased, follicle growth was promoted by rFSH and hMG under GnRHa or GnRH AN protocols.

After hCG treatment, oocyte retrieval followed by IVF was performed.
Results

- Among 83 patients, IVA was performed in 46 patients and follicle growth was found in 28 of 46 patients containing residual follicles based on the histological analyses. (no follicle growth was observed in patients without residual follicles)

- After IVF, embryos were cryopreserved at day 2.
Thawing embryo transfer was performed in 6 patients. Others were accumulating cryopreserved embryos.

3 of 6 patients became pregnant after embryo transfer.

One miscarriage
Two successful deliveries
— a male baby, 3254g;
— a female baby, 2970g).
Follicle growth from primordial to preovulatory stage takes more than 6 months.

In contrast to our expectation, we found follicle growth before 6 months after grafting.

This result suggested that our IVA method also stimulated growth of secondary follicles in grafted ovaries.
Ovarian fragmentation suppresses Hippo signaling, leading to follicle growth

Ovarian fragmentation led to changes in intercellular tension and facilitated the conversion of G-actin to F-actin.

Subsequent disruption of Hippo signaling decreased pYAP to total YAP ratios, leading to increased expression of downstream CCN growth factors.

Secretion of CCN growth factors stimulated follicle growth.
Future perspective of IVA

- In addition to POI, IVA can apply for the patients with

1) diminishing ovarian reserve
2) poor responder (IVI group in Spain)
3) aging