Case Report

Drug-free In vitro Activation for Primary Ovarian Insufficiency

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Primary Ovarian Insufficiency (POI) or Diminished Ovarian Reserve (DOR) are the two conditions that affects women leading to infertility due to the lack of follicular growth and ovulation. Egg / Oocyte donation remains an option for these patients. However, with the development in Reproductive medicine various novel technologies like Ovarian Cryopreservation, Fragmentation, in vitro activation with drug treatment or even drug free autotransplantation enables the possibility of activating the pool of primordial follicles that can lead to successful pregnancy outcomes. Here, we report a case of women with POI, in which drug free in vitro activation of follicles was performed, followed by autotransplantation, which resulted in successful pregnancy. This is first case report from India that shows the procedure works and can be tried for women with POI.

Keywords: Drug-free, in vitro activation, infertility

INTRODUCTION

Over the recent decades, age-related decreased fertility has become an increasing challenge for infertility professionals. This ‘natural infertility’ occurs due to the exhaustion of the pool of resting ovarian primordial follicles.[1] Worldwide, 1% of women suffer from primary ovarian insufficiency (POI), which leads to early exhaustion of ovarian follicles due to genetic, immunological, iatrogenic, or any ovarian pathology. They are characterised by high circulating follicle-stimulating hormone (FSH) and experience amenorrhoea before the age of 40 years. For such patients the only option is to go for egg donation. The main aim of regenerative medicine is to activate the remaining ovarian ‘reproductive gold reserve’ for such patients and also in the patients of diminished ovarian reserve (DOR).[2] In recent years, different approaches have been established to regenerate, rejuvenate or reactivate germs cells in the ovary. For the activation of dormant follicles by utilising in vitro culture of ovarian fragments treated with phosphatidylinositol 3-kinase stimulators and phosphatase and tensin homologue inhibitors.[3] Other studies have suggested that ovarian fragmentation disrupts the Hippo signalling pathway leading to ovarian follicle growth.[4] A study by Kawamura et al. merged the two concepts and demonstrated that ovarian fragmentation promoted actin polymerisation and disrupted ovarian Hippo signalling that lead to the promotion of follicle growth followed by autotransplantation.[5] Fabregues et al. further suggested that drug-free ovarian activation of follicles using fresh tissue autotransplantation could be done in POI patients.[2]

Hereby, we present a case of a patient with POI in which pregnancy was achieved using drug-free in vitro activation (IVA).

CASE REPORT

A 34 year old female had reduced anti-mullerian hormone (AMH) levels of 0.22 ng/ml. Her antral follicle count in the left ovary was 1 and in the right ovary was 2. The ovarian volume right side was 6 cc and left side was 8.2 cc. Her FSH was 11.2 mIU/ml and had a history of irregular scanty periods. Despite diverse testing including chromosomal analysis, her pathogenesis was unknown. After counseling the patient about the
procedure, the patient agreed and we further proceeded to take the approval of our Institutional Ethics Committee (ECR/842/Inst/GJ/2016/RR-20) approved the treatment, and the patient provided written and informed consent for Drug Free in vitro activation of Ovary procedure. To suppress serum gonadotropin levels before grafting ovarian fragments, pre-treatment with oral oestrogen/progesterone was given (21 day therapy).

We used a laparoscopic approach to extract the ovarian tissue. We opted to remove one-third of the right-sided ovarian cortex in an endobag. For the removal of the ovarian cortex, we used scissors and laparoscopic knife. The ovarian cortex was dissected into thin strips devoid of medullary portion with efforts to minimise any surgical trauma to the sensitive ovarian tissue, excluding the medullary portion as much as possible to minimise the surgical trauma to the sensitive ovarian tissue. Bipolar coagulation was not used on the medulla after the extraction of the ovarian cortex.

**Embryology laboratory details**

The ovarian cortex was dissected to remove residual medulla tissue. Tissue strips were then cut into small cubes (1 mm × 1 mm × 1 mm). These tiny pieces were loaded into a cannula. Histopathological analysis showed the presence of some residual follicles.

The autotransplantation was performed by laparoscopy in the same surgical procedure, and a small window (2 cm × 2 cm) was made below the right ovary and fallopian tube, and small strips of ovarian cortical tissue were transferred into the window through a modified Ryle’s tube wherein, the tube was cut before the first eye/hole at the distal end to facilitate smooth transfer of ovarian strips. The window was sutured with Vicryl 2.0. The patient was discharged on the same day of the surgery without any complications.

Pre-treatment with oestradiol/progesterone was stopped the day before ovary autografting, inducing withdrawal bleeding soon after the surgery. After initiation of withdrawal bleeding in November 2019, a baseline transvaginal sonography (TVS) revealed an antral follicle count of 3. An antagonist protocol was initiated with recombinant FSH (Folisurge and Intas Pharmaceuticals) and human menopausal gonadotropin (HMG) (IVFM, menotropin and L G Chem) and Cetrotide 0.25 mg, Merck Sereno Biopharmaceuticals, United Kingdom were added on day 7.

After 9 days of Ovarian Stimulation eight dominant follicles were noted of which six were on the right ovary and two in the left ovary were observed in transvaginal ultrasound. Recombinant human chorionic gonadotropin (ovitrelle 250; Merck, Spain) was administered, and 36 h later, oocyte retrieval was performed. Four mature oocytes were retrieved, fertilisation was achieved through intracytoplasmic sperm injection (ICSI) and 1 embryo of day 3 embryos was frozen Grade C.

In the next cycle in April 2020, stimulation was started with HMG (menotropin and Bayer Zydus Pharma Pvt Ltd). After 13 days of ovarian stimulation, seven dominant follicles were observed: four on the right side and three on the left side by transvaginal ultrasound. Dual trigger (ovitrelle 250; Merck, Spain and 0.1 mg of triptorelin acetate decapeptyl; Ferring Pharmaceuticals) was given, and 36 h later, the oocyte retrieval was performed. However, no oocytes were retrieved. Further, treatment was delayed due to COVID-19 pandemic, and the third cycle was initiated in December 2020.

An AFC of four was noted, and stimulation of the third cycle was started in December 2020. Stimulation started with injection GnRH antagonist protocol United Kingdom.

After 9 days of ovarian stimulation, seven follicles were observed (right side: five follicles: 16, 17, 19, 19 and 17 mm and left side: two follicles: 19.19 mm) by TVS. Dual trigger (ovitrelle 250; Merck, Spain and 0.1 mg of triptorelin acetate decapeptyl; ferring pharmaceuticals) was administered and 36 h later, oocyte retrieval was performed. Three eggs from the right ovary and two eggs from the left ovary were retrieved. Fertilisation was achieved through ICSI, and three good quality day 3 embryo of Grade A, A and C were transferred. There was an increase in the AMH levels too which was 0.85 ng/ml. Singleton pregnancy was established with sonographic evidence of pregnancy observed at the 5th week. Cardiac activity was established in the 6th week.

The growth parameters were satisfactory until the 9th week of pregnancy after which, the cardiac activity stopped resulting miscarriage. We still have 01 embryo of day 3 frozen from the first cycle.

**Discussion**

To our knowledge, this is the first case report from India documenting the efficacy of IVA work that has led to a pregnancy. POI patients have intermittent and unpredictable ovarian function due to the lack of follicles and ovulation. In general, in these patients, egg donation is the most widely used option to treat infertility. Thus, such development of new methods would enable patients to conceive using their own eggs. Many small studies have demonstrated that it is possible to activate dormant follicles within such ovaries and achieve pregnancy.\(^{[6,2]}\) It has been hypothesised that fragmentation of ovarian
cortex interferes with the Hippo signalling pathway and leads to the production of growth factors to promote primordial to pre-antral follicular growth, but the specific mechanism of action responsible for the activation of dormant follicles has not been elucidated.\(^7\)

In conclusion, IVA appears to be an attractive approach in the management of POI and DOR. However, it is to be highlighted that it is still an experimental procedure and larger studies are needed to ensure safety and efficacy.

**Declaration of patient consent**
The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understands that her name and initial will not be published and due efforts will be made to conceal her identity, but anonymity cannot be guaranteed.

**Financial support and sponsorship**
Nil.

**Conflicts of interest**
There are no conflicts of interest.

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