

In vitro fertilization: Information about the treatment procedure

In vitro fertilization is a series of procedures in which eggs are collected from the ovarian follicles using a transvaginal ultrasound device and fertilized, followed by embryo transfer of the fertilized eggs. In general, ovulation is induced before the egg collection. However, the eggs can also be collected in the normal natural menstrual cycle without induction of ovulation.

1. Start of ovulation induction: In principle, we would request you to visit our clinic on the 3rd day of menstruation in all the following cycles. We will measure the levels of two hormones, estradiol 2 (E2) and follicle-stimulating hormone (FSH), and perform ultrasonography. If any residual follicles are found at this time point, we will reset the system to prepare for the next cycle, without starting the in vitro fertilization cycle.

[1] Normal natural cycle: Proceed to egg collection without using clomid, letrozole, human menopausal gonadotropin (hMG) injection, or FSH injection. We will set the normal ovulation day as the egg collection day, based on your own menstrual cycle. Normally, only a single follicle shows ovulation, so that only one egg can be collected. For degenerated eggs and empty follicles, no subsequent procedure is performed. This method is indicated for women who have only one follicle development after stimulation of ovulation or those who do not achieve pregnancy by mild stimulation or in stimulated cycles. It is also indicated for post-breast cancer women in whom maintenance of low hormone levels is recommended.

[2] Mildly stimulated ovulation induction cycle: Patients are asked to take 1 tablet of

clomid or letrozole after dinner. The number of follicles and their growth rate could be increased by additional hMG or FSH injection. There is good news for those who are too busy to make clinic visits to receive the injections. Biotechnologically prepared recombinant FSH preparations are also available for self-injection, although they are expensive. Since they do not contain **luteinizing hormone (LH)**, the pregnancy rate is slightly lower than that expected with the hMG preparations. On the other hand, hMG preparations, which are produced by separating and purifying hMG from the urine of menopausal women, have been used for a long time. In recent years, due to the COVID-19 pandemic, it has become difficult to stably secure the raw material, urine, and the supply of the injectable preparations is beginning to stagnate. We use intramuscular injection formulations at our clinic. For those receiving in vitro fertilization on a self-paid basis, it is possible to receive the injections at a nearby clinic or hospital. In addition, self-injections are allowed for those who have a Medical Practitioner's License or Nursing License. The frequency and schedule of the clinic visits for injections will be set according to your convenience.

Clomid triggers the brain's response by lowering the level of the female hormone estrogen in the blood, leading to the induction of ovulation. In addition, clomid is also believed to directly stimulate the ovaries. The common side effects of this drug are headache and blurred vision, but they occur only infrequently. In some cases, clomid may cause thinning of the inner lining of the uterus (endometrium).

Letrozole, on the other hand, is a drug that has been developed to prevent recurrence of breast cancer in hormone receptor-positive patients after breast cancer surgery. Letrozole temporarily blocks the aromatic enzyme at the final step of estrogen

production from cholesterol, causing the estrogen level to decrease. While this effect is similar to that of clomid, letrozole has no direct effect on the ovaries. There are, however, data from animal experiments that raise the concern that use of letrozole during pregnancy may cause fetal malformations. In addition, in around the year 2000, it was presented at an international scientific conference in Canada that the use of letrozole for ovulation induction increased the number of fetuses with heart malformations. However, thereafter, errors in the statistical processing of these results were discovered. Because the action time is short, letrozole is eliminated from the body by the time of ovulation, even when it is taken from the 3rd to 7th day of menstruation. Therefore, there is no concern about the use of letrozole at present. Although letrozole has no noticeable side effects, its effect on the development of ovarian follicles is not seen in some patients. At our clinic, we undertake mild-stimulation in vitro fertilization, using clomid plus hMG injection as the basic treatment. However, we use letrozole, if requested, for those in whom no effect of the former stimulation regimen is seen.

[3] Ovulation induction in stimulated cycles: This is a method to grow many follicles by injecting an hMG or FSH preparation every day from the 3rd day of menstruation. It is the most commonly used method worldwide. However, attention needs to be paid to its side effects, such as development of the ovarian hyperstimulation syndrome. In order to suppress natural ovulation, the short-protocol, long-protocol, antagonist-protocol, etc. are used. However, for patients examined at the clinic every day, there is no need to suppress natural ovulation. At our clinic, the mildly-stimulated ovulation induction cycle described in [2] is used as the basic treatment. However, for patients with pituitary amenorrhea or severe polycystic ovary, method [2] may not induce the

development of follicles. For such patients, method [3] is indicated. This method may be also used at request, but method [2] is considered to be a more tolerable choice for those who do not require such treatment.

2. Testing before egg collection: As a preoperative examination before egg collection, we perform peripheral blood sampling to check whether you have anemia, inflammation, and hemostatic problems or not. In addition, we perform testing to check for hepatitis B and C, syphilis, AIDS and chlamydia infections.

After the start of ovulation induction, we perform blood sampling to examine the levels of E2, FSH, LH, and progesterone in the blood, along with transvaginal ultrasonography, in order to find the optimal day for egg collection.

Once it is decided to perform egg collection, a nasal spray is used to promote the final maturation of the eggs. Those whose pituitary glands do not respond to the nasal spray may require injections of **human chorionic gonadotropin (hCG)**.

3. Egg collection: At our clinic, egg collection is performed without anesthesia. There is no need to limit food intake before the procedure. The procedure time is about 5 to 10 minutes. However, we will ask you to come to the clinic earlier to allow us to prepare you for the procedure. Since many follicles are present in patients who have polycystic ovaries or are in a stimulated cycle, the egg collection procedure may take about 15 minutes, or more.

After the egg collection, you will be asked to rest in bed for about 20 minutes. Then, the gauze inserted after the egg collection is removed, and ultrasonography is performed to confirm that there is no bleeding in the abdomen after the procedure. If there is no problem, you will be asked to take off the surgical gown and put on your clothes that you wore to the clinic. Then, your culture specialist will explain the eggs, sperms and culture method.

Thereafter, the Director will talk to you about the transplantation.

4. How to spend the rest of the day after the egg collection: Please avoid exercising and bathing, but showering is OK. Please refrain from consuming alcohol and engaging in sexual intercourse. Please strictly follow these instructions to prevent bleeding again from the needle hole at the time of the egg collection and prevent infections. You could work, if your work is limited to desk work, although we would recommend adequate rest.

5. Embryo transfer: It is performed by either of the following methods: [1] One fresh embryo is transferred on the 2nd or 3rd day after the egg collection, the remaining embryos are cultured to the blastocyst stage, and the blastocysts are stored frozen.; [2] All embryos are cultured to the blastocyst stage, the blastocysts are stored frozen, and one embryo is transferred on the 5th day after ovulation in subsequent natural cycles. For patients who have no passage problems in the fallopian tubes and are undergoing the procedure for the first time, we select method [1]. For patients who have passage problems in the fallopian tubes or those in whom method [1] has failed once or twice, we use method [2]. For patients with anovulation or menstrual irregularity, we consider hormone replacement therapy.

For our final selection of the embryo transfer method, we also take into consideration the wishes

of the patient after the egg collection.

6. Determination of pregnancy: Pregnancy success is determined by performing blood testing on the 11th or 12th day of menstruation after the fresh embryo transfer described in 5-[1]. For the embryo transfer using frozen-thawed blastocysts in 5-[2], pregnancy success is determined by performing blood testing on the 7th day after the transfer. Since both schedules are minimal for identification of pregnancy, you will not get a pregnancy reaction even if you check it by a urine test one day before.

7. Assisted hatching for frozen blastocyst transfer: After growing into a blastocyst, the fertilized egg undergoes repeated cell division and grows in size. When the egg can no longer fit in the shell, it escapes the shell by breaking the gelatinous membrane called the “zona pellucida,” much like a chicken egg. This is called hatching. It has often been noted that the implantation rate may decrease due to consumption of energy during this escape process. Therefore, a technique for assisted hatching has been developed to take out the blastocyst from the zona pellucida in advance. Currently, fertilized eggs are taken out by making a hole in the zona pellucida using a laser beam. The guidelines of the Ministry of Health, Labour and Welfare for health insurance coverage for in vitro fertilization recommend this procedure in cases of unsuccessful pregnancy. However, to avoid repeating embryo transfer once more in consideration of the high probability of failure in advance, we have been using the assisted hatching technique for nearly 9 years in all the cases in our clinic (20 years in my clinical practice), and obtained good pregnancy outcomes. However, if you do not want to undergo this method, please do indicate it to us.

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