

## content

**"Double Freeze" ICSI Success Delivery Introduction**  
**Case Studies and Literature Review**

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**Abstract**

**Objective:** To investigate the clinical effect of ICSI on cryopreserved oocyte and sperm.

**Methods:** Report a case of intracytoplasmic sperm injection (ICSI) patient through successive cryopreserved oocytes and sperm, discussed the results together with literature review.

**Results:** Patients with successful cesarean delivery of a healthy baby girl after a successful fresh embryo transplantation by using the fertilized cryopreserved oocyte and sperm after ICSI

**Conclusion:** ICSI on cryopreserved oocyte and sperm is a reliable and efficient method on special case, such as sperm collection failed patients at the oocyte retrieval day.

**Keywords:** Oocyte; Sperm; Cryopreservation; Vitrification; Intracytoplasmic sperm injection (ICSI); Embryo transfer (ET)

**Table 1** Incident and outcome of pregnancy

Time	Incident	Outcome
2009.03	Ectopic pregnancy	Left fallopian tube incision laparoscopic surgery
2011.04	Bilateral tubal dredge and bilateral laparoscopic ovarian drilling	Not pregnancy
2011.06	Spontaneous abortion	Abortion
2012.10	GnRH-a long protocol COH	12 Eggs, cryopreservation by vitrification

CLC: R715.2

Since 2006 China's first but also the second case in the world of "three frozen" test-tube baby was born success, capturing the freeze-thaw human oocytes technical difficulties, significant progress indicates that China assisted reproductive technology has made, and now one of our hospital's PCOS (polycystic ovary syndrome) patients experienced "double freeze" test-tube baby technology successful delivery.

**1. Clinical Data**

Patient Xu, 27 years old, on June 19, 2012 with "spontaneous abortion after more than a year is not pregnant," the main complaint admitted. The woman married for three years, conjugal, normal sex life. March 2009 had an ectopic pregnancy the line "left fallopian tube incision laparoscopic surgery", June 2011 first trimester spontaneous abortion (not curettage), more than a year after abortion is still not pregnant. Usually due to the rules of menstruation, period 38-60 days, there is a history of PCOS, oral Diane-35 and Chinese medicine treatment. March 2011, HSG examinations showed "the right of tubal less smooth, left fallopian tube may ampulla obstruction."

In April 2011, other hospital for "bilateral tubal dredge and bilateral laparoscopic ovarian drilling", after ovulation, not pregnancy. Male semen "mild asthenozoospermia", the woman hormone-based measurement: FSH: 6.68IU / L, LH: 13.16IU / L, E2: 119.5pmol / L, T: 1.14 nmol / L, PRL: 5.92 ng / ml. Clinical diagnosis: secondary infertility, tubal obstruction on the left and right salpingitis, PCOS. Patients with short-acting long down-regulation program, using short-acting drugs Decapeptyl and recombinant human follicle-stimulating hormone (Gonal-f). On October 23, 2012 to give HMG intramuscular injection 75 ml, after HCG trigger for 36 hours, retrieval 12 eggs, no obvious discomfort and bleeding.

2013.03	ICSI on 10 cryopreserved oocyte and sperm	2 Fresh embryos transplantation, 6 embryos cryopreservation
2013.12	Pregnant 40 W + 4	Cesarean delivery a healthy baby girl

The spouse Masturbation failed on the oocyte retrieval day and was not willing to testicular sperm retrieval by puncture, followed by oocyte cryopreservation of eggs, sperm cryopreservation parallel backup. March 26, 2013 the patients undergoing ICSI thawed frozen eggs, on the thawed oocytes day, spouse masturbation sperm fail again, then thawed frozen sperm, and thawed oocytes within intracytoplasmic sperm injection technique. Total ICSI 10 oocytes, were normal fertilization, 8 embryo transplant eligible embryos, including six high-quality embryos, on March 29, 2013 Day 3 transplant two fresh embryos (grade 8 CI and 9 CII level), six embryos frozen. April 26, 2013, the patient diagnosed with intrauterine pregnancies, survived. December 14, 2013 pregnant 40 W + 4 term cesarean delivery a baby girl, weighing 3150 g, Apgar score 10, in good health (Table 1).

## 2. Discussion

Gamete and embryo cryopreservation has become the field of conventional in vitro fertilization embryo transfer (IVF-ET) technology, the success of frozen gametes technology can increase efficiency, improve cycle transplant patients and cumulative pregnancy rate, reduce their physical suffering of patients, as well as economic and emotional burden[1,2]. "Three freeze" technique, the frozen embryos in the field of reproductive technology is quite mature, but thaw human oocytes and sperm technology is not yet mature, is still at an initial stage, is the forefront of the field of assisted reproductive technology. In terms of cryopreserved oocytes, vitrification procedure slow compared to freezing, the cells cannot produce a solution and frozen ice crystals when frozen, reducing cell damage, its economic, efficient and does not affect the embryo bed rate and better preserved embryo developmental potential, improve the clinical pregnancy rate is conducive to successful establishment of pregnancy, but also more suitable for preservation of human ovarian tissue, has broad application prospects [3-5]. However, freezing and thawing process itself may have the structure and function of oocytes have an impact, especially for mature oocytes may disrupt the cytoskeleton, causing the spindle distortion and fracture, causing chromosomal dispersion, and increase embryo aneuploidy Body risks, how to thaw fertilized oocytes to the appropriate temperature, as well as improve the zona pellucida hardens after freezing is still problems we faced [6-8]. For patients with a spouse in this case, the use of low sperm programmed freezing method. Slow freezing procedure is difficult to avoid damage caused by ice crystal formation, the relocation of excessive intracellular water, increase cell osmotic pressure, can cause toxic reactions. While freezing sperm recovery process may cause sperm outer membrane rupture thereby affecting the survival of sperm, the acrosome membrane damage can be reduced by acrosin activity, structural changes of mitochondria weakened sperm motility and oxygen resistance capability [9]. After frozen-thawed

sperm DNA damage determines the embryo quality, and whether sperm vitrification still a large number of sample studies can improve sperm fertilizing capacity.

The patients were simultaneously thawed oocytes and sperm, ICSI and implementation of high-quality fresh embryo transplant to be successful pregnancy and childbirth, not only with frozen gametes method, but also with the formation of the number of good quality embryos related. The patients were treated with oocyte vitrification method to be successful, the patient may become unable to obtain sperm oocyte day remedies. For cancer patients before chemotherapy or planned childbearing women, the vitrification of oocytes quality to preserve fertility is valid choice, but it remains a technical challenge [10-13]. And select the appropriate method for freezing, frozen carrier concentration and the best protection is still the future of frozen sperm cryopreservation scarce research focus, combined with ICSI can improve small, weak, or no sperm fertility patient outcomes.

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