

# Livebirth Derived from In Vitro Matured Oocytes

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## Abstract

The authors presented a case report of a woman who suffered from primary infertility due to anovulation. She was diagnosed to have polycystic ovarian syndrome (PCOS) and was treated by ovarian stimulation with conventional technique, such as clomiphene citrate alone, the combination of clomiphene citrate and metformin and the combination of clomiphene citrate with gonadotropins. Nevertheless all of them resulted in no response. Therefore, in vitro maturation of oocyte (IVM) was started and followed by intracytoplasmic sperm injection (ICSI) of 22 oocytes. 14 embryos developed and three of them were transferred back to the uterine cavity. She conceived and the ultrasonography revealed twin pregnancy at the 8-weeks gestation. She gave birth at the 31 weeks gestation in Siriraj Hospital. The newborns were admitted in the hospital for 24 days for phototherapy in treating jaundice which was due to prematurity. Then both of them were discharged healthily.

**Keywords:** In vitro maturation, polycystic ovarian syndrome, gonadotropin priming

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**I**n vitro fertilization (IVF) has been widely used and studied for almost 30 years. The high cost of the IVF procedures prevented many couples from continuous treatment. Besides that some complications, such as ovarian hyperstimulation syndrome (OHSS) may occur and threaten the couples. Therefore patients who are responded well to the gonadotropin stimulation, for instance, patients with polycystic ovarian syndrome, have a significant risk to develop OHSS. Several studies show that lots of ovarian stimulation protocols were implemented to minimize such a complication. In vitro maturation (IVM) of human oocytes is one of the novel techniques to solve the problem.

This innovative idea was first reported by Cha KY in 1991. It has been proven that immature oocytes retrieved from 2- to 8-mm diameter antral follicles within either stimulated or unstimulated ovaries can grow to maturity in vitro in 24 to 48 hours.<sup>1,2</sup> The oocyte maturation rate at 48 hours varies from 54.8% to 84.3%.<sup>3-5</sup> Otherwise the pregnancy rate is still low though some studies have reported a high pregnancy rate as high as 37%. In Thailand, IVM is still a new technique.

## CASE REPORT

A 30 year-old Thai woman came to Siriraj Hospital for treatment of infertility. She has tried to conceive naturally for 10 years. Her menstrual pattern was oligomenor-

rhea. The physical examination was normal. She had no signs indicating hirsutism or acanthosis nigricans. No acne could be identified. She weighed 60 kg and was 163 cm in height. BMI was 24.46 kg/m<sup>2</sup>. The pelvic examination revealed a normal uterus and transvaginal ultrasonography revealed a polycystic appearance of both ovaries. She was diagnosed as polycystic ovarian syndrome, according to the Rotterdams criteria. The blood examinations for sexually transmitted disease screening were negative. CBC, fasting blood sugar (FBS) as well as the basal hormone levels of FSH, LH, estradiol and prolactin were all in normal range. The male semen analysis was also normal. Ovarian stimulation and intrauterine insemination (IUI) was introduced to this couple. Unfortunately, no dominant follicle was identified after the stimulation with either clomiphene citrate alone or the combination of clomiphene citrate with metformin and gonadotropins. Therefore, the treatment with IVM of oocytes was introduced to the patient. Counseling and details of the treatment were provided. The couple accepted and decided to start this technique.

150 units of rFSH (75 IU, Gonal-F®; Serono, Swiss) was commenced from the third day of the menstrual cycle, and was continued for 4 days. Pelvic ultrasonography was repeated on the seventh day and revealed approximately 33 antral follicles with variable size, ranging from 3 mm to 8 mm in diameter. The endometrial thickness was 7.3 mm. Since then Estrofem® 8 mg/day was administered vaginally. The patient then had 10,000 IU of hCG (Pregnyl®; Organon, Holland) intramuscularly on the eighth day. Oocyte retrieval was performed 36 hours after hCG injection by ultrasound-guided transvaginal aspiration with a 19 gauge single-lumen aspiration needle (K-OPS-7035-RWH-ET,

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Cook®; Ireland) under the aspiration pressure of 80 mmHg. Totally, 35 immature cumulus-oocyte complexes were retrieved.

All the cumulus-oocyte complexes were stabilized in the specially prepared IVM media, LAG medium (MediCut®; Denmark), in an incubator at 37°C under an atmosphere enriched to 6% CO<sub>2</sub> for 2 hours and then were transferred to the final IVM Maturation medium. After 28 hours of incubation, 13 oocytes developed to a mature oocyte, metaphase II. All the mature oocytes were inseminated by microinjection (ICSI) with the husbands sperm in the same day. After 52 hours maturation 9 more oocytes grew mature and were inseminated.

After 3 days of incubation, the 3 best embryos in the cleavage stage were transferred back into the endometrial cavity and another 6 embryos were cryopreserved. Endometrial thickness at the day of embryo transfer was 8.2 mm. Progesterin (8% crinone®, Serono, Swiss) was prescribed for luteal support. The β-hCG level at 14 days after embryo transfer was 1,029 mIU/ml. Transvaginal ultrasonography was performed one week later and 3 gestational sacs were identified in the uterine cavity. Unfortunately, at the eighth weeks gestation, one fetal demise was found. Only two fetuses with dichionic diamniotic sacs remained. Anomaly scan with ultrasonography was performed at the 18- weeks gestation. No anomaly could be identified. The growth scan was repeated every time of antenatal care and the fetuses had similar normal growth. The placenta was fused to be one and confined at the posterior wall of the uterus. Regrettably, preterm labor was diagnosed and failed to be inhibited at 31 weeks of gestation. The twins were in the transverse lie, so cesarean section was performed. Two male infants were delivered with a birth weight of 1,640 gm each. The APGAR scores were 9 and 10 for the twin A; 7 and 9 for twin B. Both babies had jaundice due to prematurity and were treated with phototherapy. One of the babies (twin B) had transient hypoglycemia and transient tachypnea of newborn, both of them were admitted in the hospital for 24 days and discharged healthy.

## DISCUSSION

In the case of clomiphene citrate resistance, either ovarian stimulation with gonadotropin or laparoscopic ovarian drilling (LOD) are the traditional second line strategies.<sup>6</sup> Combination of metformin and clomiphene citrate is recognized to be the new second line strategy. Stimulation with gonadotropin is costly, time consuming and putting the patient at risk of OHSS. On the other hand, the potential risk of intraoperative risk and postsurgical periovarian adhesion after LOD was considered to be counseled. With resistance to the entire noninvasive regimens IVM which can avoid ovarian hyperstimulation in conventional IVF should be considered. Therefore, severe OHSS, the rare but terrible iatrogenic complication can be prevented. Women with polycystic ovarian syndrome with clomiphene citrate resistance become the proper candidates for IVM.

According to previous studies, FSH and LH priming may be beneficial for PCOS women on several aspects. In this case the authors used 150 units of FSH for priming of the follicles because several studies corroborated that FSH priming of follicles resulted in an efficient recovery.<sup>1,7</sup> In this case 35 oocytes were obtained. It can be postulated that FSH stimulation can enlarge the follicle

size which makes it easy to be aspirated. In addition to FSH, LH injection before retrieval can induce expansion or scattering appearance of cumulus cells which facilitates detachment and expulsion of the cumulus-oocyte-complex, leading to successful oocyte retrieval.

Both FSH and LH<sup>3,5</sup> cannot only promote the oocyte retrieval, but also improve its developmental competence. FSH can stimulate steroidogenesis of the cumulus cells and the oocyte RNA and protein production<sup>8</sup> which are essential in cytoplasmic maturation of oocyte for the early embryogenesis and thus normal fetal development.<sup>9</sup> Nevertheless the effects of FSH on the promotion of the oocyte maturation rate, fertilization rate and embryo development are still controversial.<sup>8,10</sup> On the contrary, immature oocytes with dispersed cumulus cells retrieved in LH priming cycles have a higher maturation potential and better embryonic development compared with those with compacted cumulus cells.<sup>11,12</sup> In this patient the oocyte maturation rate was 37.14% and 62.86% in 24 and 48 hours respectively, which is comparable with previous studies (36-55.9%).<sup>3,5</sup>

Hormonal priming is one of the important steps to achieve the successful IVM, and appropriate follicular aspiration technique is another crucial process. Small follicles need finer calibre needles with less bevel length. The 19 gauge single-lumen aspiration needle that we used in this case is considered suitable. The ideal aspiration pressure was suggested to be around 50-80 mmHg; as the higher suction pressure was supposed to denude the immature oocyte of granulosa cells. However studies reported by Chian<sup>4</sup> revealed good results with the suction pressure of 80-100 mmHg which was used in routine IVF cycles. More studies should be conducted to find the most appropriate pressure ranges to recover the most good quality oocyte-cumulus-complex.

To obtain embryos with good quality, proper culture medium is acquired. The optimal culture condition should certainly be similar to that of the follicular fluid in vivo. In addition to FSH and LH which are the hormones needed for oocyte development in vivo, so the medium was supplemented with maternal serum; because it can significantly increase the rates of maturation, pregnancy and implantation compared with human serum albumin (HSA). It was proposed that other important substances, for instance, the epidermal growth factor or insulin-like growth factor-1, which are thought to be indispensable for cytoplasmic maturation are used in the maternal serum<sup>13</sup>. With ideal conditions oocyte maturation can achieve the highest potential; and the high maturation rate is beneficial for higher fertilization rate and pregnancy rate.

IVM has convincing advantages; however, the possibility of increased incidence of congenital anomaly in an ART baby is still a concerned point. The observed odds ratio for any congenital abnormality was 1.42 for IVM compared with controls.<sup>14</sup> Ultrasonography should be performed to diagnose anomalies. Anyway comparison of IVF and ICSI elucidated that IVM is not associated with any additional risk.<sup>14,15</sup> Neurological abnormality among survivors associated with one fetal demise usually occurs in the case in which fetal demise happens after the first trimester.<sup>16</sup> Fortunately, in this case, the fetal death occurred during the first trimester and the ultrasonography found no abnormalities in the remainders.

Multifetal gestation is a common problem in IVF. Nowadays transferring a single blastocyst instead of multiple cleavage embryos makes it possible to raise the

implantation rate as well as the pregnancy rate<sup>17</sup>, and thus reduce the iatrogenic multiple pregnancy rate. It may be preferable to transfer blastocysts. However, few studies<sup>18-20</sup> have described such successful results in IVM. Immature oocytes need a lot of unknown mechanisms and chemicals to achieve the perfect simultaneous maturation of both nucleus and cytoplasm. More researches are needed to explore the mysteries in oocyte and embryo culture which is the key factor to support the blastocyst development. Therefore most of the IVM studies have been limited by replacing only cleavage stage embryos.<sup>5,11,21,22</sup> In vitro blastocysts formation from immature human oocytes is a challenging subject for further study.

IVM is a new procedure with reasonable cost and appropriate time of treatment, short ovarian stimulation, and less complications. It is beneficial for PCOS patients who resist to medical ovarian stimulation. Although the fertilization rate and pregnancy rate of IVM are still lower than conventional IVF, in the near future the improvement of the technique and the medium will raise its potential to replace the IVF protocol in PCOS. In addition to the aforementioned, single good quality embryo transfer should be encouraged to avoid the multiple pregnancies in young women.

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