

# Birth after human chorionic gonadotropin-primed oocyte *in vitro* maturation and fertilization with testicular sperm in a normo-ovulatory patient

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## ABSTRACT

In this report, we present a case of *in vitro* maturation (IVM) with surgical retrieved testicular sperm in a normo-ovulatory female. Human chorionic gonadotropin-primed IVM, testicular biopsy for sperm retrieval and intracytoplasmic sperm injection with fresh sperm were performed. Fourteen cumulus-oocyte complexes were obtained in germinal vesicle or metaphase I stage, eight oocytes reached metaphase II, seven presumptive zygotes were obtained, and three cleavage stages embryos in day 2 were transferred producing a singleton pregnancy. A single healthy newborn was obtained. Our results suggest that IVM may be an alternative for *in vitro* fertilization in normo-ovulatory women even if surgical retrieval of sperm is needed. Further research is required to depict contributing factors to the success of IVM in indications different from polycystic ovaries syndrome and the role of male gamete.

**KEY WORDS:** Azoospermia, germinal vesicle, *in vitro* maturation, intracytoplasmic sperm injection, mild approach, surgical retrieval of sperm

## INTRODUCTION

*In vitro* maturation (IVM) is a technique described in the 90s primarily as alternative technique to conventional stimulation for patients with polycystic ovaries syndrome (PCOS) because the decreased risk of ovary hyperstimulation.<sup>[1]</sup> However, IVM has additional benefits such as a reduced cost compared to regular *in vitro* fertilization (IVF) procedures and represents a friendly approach for female patients.

Recent reviews of the literature confirmed that IVM is inferior to conventional stimulation in terms of pregnancy and take-home baby rates.<sup>[1,2]</sup> This is why IVM is considered as an experimental procedure according to the American Society for Reproductive Medicine<sup>[3]</sup> and its application is limited in the United States. Despite IVM is routinely applied in some European countries and it is a flagship for the mild approaches movement.

One potential application is the use of IVM as a less expensive alternative to stimulated IVF in case of severe male infertility. The experience of

using IVM with non-ejaculated sperm is limited, case reports of pregnancies are published from sperm obtained by testicular biopsy,<sup>[4]</sup> testicular fine-needle aspiration,<sup>[5,6]</sup> percutaneous epididymal sperm extraction,<sup>[7]</sup> and microtesticular sperm extraction.<sup>[8]</sup> Nevertheless, some of the reports used a low stimulation including low doses of follicle-stimulating hormone (FSH), menotropins, or clomiphene citrate that constitute “mild stimulation” cycles and not IVM.

In our private center in Leon Mexico, we started to offer IVM routinely in 2007.<sup>[9]</sup> In

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this report, we describe a case where human chorionic gonadotropin (hCG)-primed IVM was performed for a male infertility indication using a testicular sperm sample from a patient with nonobstructive azoospermia.

## CASE REPORT

### Patients

A 28-year-old male with nonobstructive azoospermia with normal karyotype, no Y chromosome microdeletions, or common mutations in *CFTR* gene and FSH 12 mUI/mL, luteinizing hormone (LH) 9.0 mUI/mL, and testosterone 300 ng/mL. Female partner 34 years with primary infertility, normal menstrual cycles, basal FSH 7.4 mUI/mL, LH 2.5 mUI/L, E2: 22.5/L, testosterone 0.2 ng/mL, 14 follicles at antral follicle count, and endometrial thickness of 9 mm.

Taking into account that no female factors were detected, and the prognosis was good, we decided offer IVM as treatment option to the couple. The couple had chosen IVM for their desire of a lower cost in IVF procedures. The local review board approved the procedure and present report. The couple provided informed consent for the IVF/biopsy procedures.

### *In vitro* maturation procedures

A transvaginal ultrasound scan on day 3 revealed ten antral follicles. On day 7 of the cycle, a follow-up of follicle growth was performed every 2 days. When one follicle reached 12–14 mm and endometrial thickness was 7 mm, the patient received a single intramuscular injection of 250 µg of recombinant hCG (Ovidrel, Merck Serono, Switzerland). Thirty-six hour after hCG injection, oocyte retrieval was performed under patient intravenous (IV) sedation using a 17-gauge ×20 single lumen needle (Kitazato Oocyte Pickup Needle). Fourteen cumulus-oocytes complexes were retrieved, cumulus cells were removed using hyaluronidase, no mature (metaphase II) oocytes were found. Immature oocytes (germinal vesicle or metaphase I oocytes) were cultured in IVM media (SAGE) supplemented with 0.75 IU/mL of recombinant FSH (rFSH) and 0.75 IU/mL of recombinant LH. After 24 h of culture oocytes were examined, eight oocytes reached metaphase II.

Testicular biopsy was performed under IV sedation 1 day after oocyte capture, fresh spermatozoa obtained by testicular biopsy. The testicular tissue was minced into small pieces using two scalpels in human tubal fluid (HTF)-HEPES, the supernatant was centrifuged at resuspended in fresh HTF until intracytoplasmic sperm injection (ICSI). ICSI was performed by regular procedures. Injected oocytes were transferred to Global medium (LifeGlobal, USA) incubated at 37°C in 6% CO<sub>2</sub>. After 18 h, seven presumptive zygotes with two pronuclei were obtained. Embryos were culture

in 20 µl droplets of Global medium at 37°C in 6% CO<sub>2</sub>, three cleavage embryos in day 2 were transferred.

A singleton live birth at 37 weeks was obtained with a weight of 2700 g and length of 50 cm, and Apgar score was 9/9/9.

## DISCUSSION

In this report, a live birth from an hCG-primed IVM using testicular sperm is reported, this as part of routine offer of IVM to selected patients in our clinic in Mexico. Because pregnancy rates are lower that for conventional IVF,<sup>[1,3]</sup> it is difficult to introduce this procedure in private practice where patients cover completely with the cost of assisted reproduction procedures. However, IVM offers several advantages for the case of male infertility such as a friendly procedure for a female partner, the absence of risk related with ovary stimulation in otherwise healthy women, and the reduction in costs of treatment.

The published experience of IVM using testicular sperm is quite heterogeneous, and in some reports, the stimulation is more intense than simple hCG priming,<sup>[5,6,10]</sup> contradicting the main benefit of IVM. On the other hand, apparently IVM in normo-ovulatory women and pregnancy rates are lower than in PCOS patients.<sup>[2]</sup> The biggest experience of ICSI with testis biopsies from non-obstructive azoospermia in normo-ovulatory women using rFSH + hCG indicated that pregnancy rates are similar.<sup>[10]</sup> However, in PCOS patients using only hCG lower fertilization and pregnancy rates are reported.<sup>[11]</sup>

In the present case, pregnancy was obtained at the first IVM attempt; but further research is required to understand factors impacting the results of IVM, such as hormonal priming, ovary function, and the etiology of azoospermia when IVM oocytes are fertilized using testicular spermatozoa. Summarizing IVM may be an option for normo-ovulatory women in cases of male infertility that requires ICSI with sperm obtained from testicular biopsies.

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### Conflicts of interest

REPA has received a research fellowship from IBSA Foundation.

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