

## Healthy Birth Resulting from in Vitro Matured Oocyte Fertilized with Testicular Spermatozoa

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### Abstract

This case report describes a live birth resulting from intracytoplasmic sperm injection (ICSI) of spermatozoa retrieved by microdissection testicular sperm extraction (micro-TESE) into in vitro matured oocyte produced from controlled ovarian hyperstimulation cycle. A total of 11 oocytes (4 atretic and 7 immature oocytes) were retrieved. Following IVM, all immature oocytes had matured. A total of 5 oocytes were fertilized after ICSI with the husband's micro-TESE spermatozoa and 2 embryos were transferred into the uterus on day 2. A healthy girl weighing 3650 g was born at 38 weeks of gestation.

**Keywords:** In vitro maturation; Immature oocyte; ICSI; Testicular biopsy; Live birth

### Introduction

The degree of sperm maturation can affect its fertilization competence when fertilized with oocytes matured in vitro after ICSI, the percentage of IVM oocytes achieving normal fertilization following ICSI was significantly lower using the testicular spermatozoa than the ejaculated spermatozoa [1,2]. The published experience on pregnancies derived from IVM oocytes fertilized by spermatozoa retrieved from the testis remains limited to only some case reports and a very small series of treatments. We report a case of successful pregnancy and healthy birth resulting from in vitro matured oocytes fertilized with testicular spermatozoa.

### Case Report

A couple consulted our centre of reproductive Biology for primary 8 year infertility. A 41-year-old man had no medical or surgical history except non obstructive azoospermia checked in two sperm analysis. The clinical examination of the testes was normal. The follicle stimulating hormone level was 13.1 IU/L (reference values 1.4-11.2 IU/L). The constitutional karyotype was 46, XY. His wife is 32 years, the results of her infertility investigations were normal except the biochemical features of polycystic ovaries (PCO) (FSH = 6, 81 IU/L, LH=14, 27 IU/L, PRL= 14 IU/L). The couple was counselled to undergo therapeutic testicular biopsy on the day of ovum pick up. The cycle treatment with controlled ovarian hyperstimulation was started with 225 IU of recombinant FSH (Gonal-F®; Serono Nordic, Solna, Sweden) after downregulation with triptorelin LP (Decapeptyl®, Ipsen Pharma) [3]. Transvaginal oocyte retrieval was performed 36 h after a human chorionic gonadotrophin (Ovitrelle®; Serono Nordic, Solna, Sweden) administration. We retrieved 11 cumulus-oocyte complexes. We removed a cumulus cells carefully by a brief exposure to hyaluronidase (HyaseTM171, Vitrolife, Sweden) and mechanical denudation with a fine pipette. We assessed the oocytes for their meiotic stage according to the presence or absence of GV or first polar

body (PB1). The MI state of the oocytes was defined by the absence of a polar body and GV nucleus. Four oocytes were atretic and 7 had MI state. The immature oocytes were incubated in our routine culture medium (G1; Vitrolife, Sweden), at 37°C, 6% CO<sub>2</sub> in air. No oocyte became mature the day of ovarian pick up (day 0). Consequently, the testicular biopsy was not performed. After 24 hours of oocyte incubation (day1), all the MI oocytes extruded their polar body (rescued in vitro-matured metaphase II). We recovered a husband's spermatozoa by microdissection testicular sperm extraction (micro-TESE) [2]. We divided a sample using 2 needles and we examined them at a magnification of 400X in the inverted microscope for the presence of sperm cells. We found a few immotile spermatozoa. Discontinuous minidensity gradients (containing volumes of only 0.3 mL of different density gradient materials (45% and 90% of Sil Select Stock® Fertipro, Belgium) were used to separate spermatozoa and spermatids. After centrifugation (300 rpm), the pellet was gently resuspended in HSA medium at 37°C, 5% CO<sub>2</sub> until ICSI. All MII oocytes were microinjected with testicular immotile spermatozoa according to standard protocols [4]. Fertilization was confirmed 18 hours later. Five oocytes became fertilized and four of them cleaved but only two of them produces good score embryos which were transferred on day 2 at the 4-cell stage. This embryo transfer resulted in a normal intrauterine singleton pregnancy. After 38 weeks of gestation a healthy girl, weighing 3650 g (Apgar 9, 10, 10), was delivered by caesarean section.

### Discussion

Approximately 5%–20% of human oocytes collected from women after stimulation of the ovaries for IVF fail to resume meiosis in vivo and are thus retrieved in immature stages like metaphase I (MI) or germinal vesicle (GV) [5]. The immature oocytes underwent an in vitro maturation process (IVM) using or not specific culture media. Different incubation periods ranged from 2 to 24 hours seem suitable to enable proper nuclear and cytoplasmic maturation (Metaphase II (MII) stage) and the MI oocytes that extruded their polar body, named as rescued in-vitro matured MII oocytes [6]. Several studies have compared the development potential of these rescued IVM-MII

oocytes to matured MII oocytes and their conclusion were as a lower normal fertilization rate with abnormal embryonic development and lower implantation rate than in vivo matured oocytes [1,7,8]. Development to term is limited to rare cases. Therefore, sperm testicular extraction to inject these rescued in-vitro matured MII oocytes was much discussed. In fact, the spermatozoa extracted from the testes were immature and the comparative studies after ICSI with ejaculated and testicular sperm showed a lower percentage of new live births per embryo transferred, and twice as many spontaneous miscarriages as after the use of surgical sperm [4,9]. In addition, the baseline incidence of sperm chromosomal abnormalities is higher in the testis of infertile individuals [10]. But in line with our findings, a several analysis of surgical sperm collected in azoospermic patients concluded that ICSI outcomes were not affected by the sperm origin [11,12]. The age of the female partner and oocyte quality has been shown to exert a substantial influence on the success of ICSI treatment using testicular spermatozoa [13,14]. In our case, the young age and the PCO profile of the patient seem to be a good prognostic factor for a successful attempt despite the double immaturity problem of gametes.

## Conclusion

We may conclude that the use of rescued-IVM MI oocytes can be beneficial in order to obtain pregnancy in younger stimulated patients without MII oocytes. Despite their microinjection with immature spermatozoa, fertilization of in vitro matured MI oocytes can result in normal embryos, pregnancy, and the delivery of a healthy child.

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