



Opinion

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The Complex Infertility Treatment: Optimum Time for Oocyte Vitrification as Indicated by Meiotic Spindle, with Thawed Oocytes Fertilized by Intracytoplasmic Sperm Injection

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To Cite This Article: J Masata, O Tepla, S Jirsova, M Moosova, K Rezabek, Irena Kratochvilova. The Complex Infertility Treatment: Optimum Time for Oocyte Vitrification as Indicated by Meiotic Spindle, with Thawed Oocytes Fertilized by Intracytoplasmic Sperm Injection. *Am J Biomed Sci & Res.* 2022 - 15(5). *AJBSR.MS.ID.002152*. DOI: [10.34297/AJBSR.2022.15.002152](https://doi.org/10.34297/AJBSR.2022.15.002152)

Received: February 28, 2022; **Published:** March 07, 2022

Introduction

Here we report the pregnancy of 38 years old women with low ovarian reserve whose spouse originally had no living sperms. The IVF/ICSI cycle was a first-choice treatment. In that case, collected oocytes had to be vitrified and thawed before fertilization. Oocyte polar body and optically birefringent meiotic spindle were visualized by polarized light microscopy and their states and relative positions were used as an indicators of full oocyte maturation, i.e., readiness for vitrification. According to our opinion this strategy enabled to vitrify fully mature (MII phase) oocytes, which were after thawing prepared for ICSI. Thawed oocytes were finally fertilized by intracytoplasmic sperm injection (ICSI) [1-3]. The 42 years old man spermogram was improved by Tamoxifen treatment.

A 38-year-old Caucasian woman (G 0/P0, body mass index: 22.50) and her husband were investigated in the reproductive medicine unit of a university hospital for 2-year primary infertility in 2019. She had no personal history of any disease or cancer, no surgery, and took no medication. Low ovarian reserve was found on ultrasound. Laboratory workup confirmed low ovarian reserve (day 2 FSH 8.11 IU/l, AMH 0.6 ng/ml). The patient's husband was a 43-year-old Caucasian man with no history of excess alcohol

consumption, no exposure to toxicants and no ongoing medical treatment. He had neither genital disease nor varicocele and no history of testicular injury. No available immunological cause of the patient's fertility was found. The couple decided to undergo IVF/ICSI cycle as a first-choice treatment. On 24 January 2021 the first IVF cycle took place (short protocol rFSH 300 IU + antagonist were used). Two oocytes were collected. No vital sperms were available for fertilization, and the partners refused to use donor sperms; vitrification of oocyte was offered. Because the patient was more than 36 years of age, evaluation of meiotic spindle by polarized light microscopy was performed, and in one case the spindle was barely visible. 6 hours after meiotic spindle evaluation both oocytes were cryopreserved by vitrification. In the next cycle (same stimulation protocol) two oocytes were again collected (no sperms available). The evaluation of meiotic spindle was performed in both cases. In one case the spindle was barely visible, while the other oocyte had visible meiotic spindle close to polar body. 4.5 hours after meiotic spindle evaluation both oocytes were cryopreserved by vitrification. Even in this case, both oocytes had time for maturation before vitrification. Afterwards the patient's husband took Tamoxifen tbl. 1-0-0 for two weeks, which resulted in spermogram improvement. Fresh sperms were obtained from the patient's husband. That day



at 9:10 four oocytes were thawed, and meiotic spindles evaluation showed M2 maturity in two cases. In one oocyte the visibility of the meiotic spindle was not perfect, in one oocyte the meiotic spindle was perfectly visible. The same day at 11:30 both oocytes were fertilized by ICSI. Embryo transfer 8/1 gr. 1, in two cases the embryo development was stopped at day 3. Two weeks later a pregnancy test was positive and clinical pregnancy was diagnosed by ultrasound (now 30th week of gestation) [4,5].

The presented couple infertility was a combination of female (low ovarian reserve) and male (no vital sperms) factors. The IVF/ICSI cycle was a first-choice treatment. The male spermogram was improved after Tamoxifen application. To match the improved spermogram with highest quality oocytes, the 38 years old female oocytes had to be vitrified. The time of oocytes vitrification was specified by meiotic spindle state and its relative position to the polar body. According to our opinion this strategy enabled to

vitrify fully mature (MII phase) oocytes, which were after thawing prepared for ICSI. Finally, healthy baby girl was born.

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