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# Successful Pregnancy and Delivery Following Intracytoplasmic Injection of Frozen-Thawed Nonviable Testicular Sperm and Oocyte Activation With Calcium Ionophore

Case Report

ALI AHMADY*† AND ESSAM MICHAEL*

*From the *Astra Fertility Clinic, Mississauga, Ontario, Canada, †Dr Ahmady is now with University Fertility Centre, Torrance, California.*

Several pregnancies have been reported using frozen-thawed testicular sperm (Fisher et al, 1996; Podsiadly et al, 1996; Khalifeh et al, 1997), stressing the role of sperm motility for successful outcome and lower rate of fertilization associated with immotile sperm (Nagy et al, 1998; Shulman et al, 1999). However, there is no report describing outcome when nonviable testicular sperm is used for intracytoplasmic injection (ICSI). In this communication, we report successful pregnancy and full term delivery following injection of nonviable frozen-thawed testicular sperm into the cytoplasm of a mature oocyte followed by oocyte activation with calcium inophore.

## Case Report

This study was approved by our institutional board. A 36-year-old patient and her 34-year-old husband, with 2 years history of primary infertility, were referred to our center for treatment. The patient was a poor responder. Her husband had suspected obstructive azoospermia. Testicular biopsy was preformed before the initiation of the cycle to ensure having sperms on hand. Retrieved sperms were frozen by addition of glycerol base cryoprotectant (Fertipro, Beernum, Belgium) and cooling in liquid nitrogen vapor for 30 minutes. Occasional motile sperms were seen before freezing. The frozen sample was thawed by removing it from the storage and immersing in 37°C water bath for 4 minutes.

Ovarian stimulation was performed using microflare protocol; namely, diluted leuprolide acetate, a gonadotropin releasing hormone agonist (GNRHa), and human menopausal gonadotropin (HMG). Daily dose of 0.05 mg Lupron subcutaneously bid and 300 IU HMG were administered starting day 2 of the cycle for 11 days. Follicular development was monitored by serial estradiol monitoring and transvaginal ultrasound. Human chorionic gonadotropin (hCG), 10 000 IU, was administered when optimal follicular development was achieved. Oocyte retrieval was performed by transvaginal ultrasonographic guided approach, 35 hours after hCG administration. Only 2 oocytes were retrieved. Only 1 oocyte was found suitable for ICSI.

A single sperm curling (SSC) test was used to evaluate viability of sperm (Ahmadi and Ng, 1997). SSC medium was prepared by adding 0.5 mL Hepes buffered medium to 1 mL distilled water to obtain osmolarity around 100 mOsm/kg. A single spermatozoon was exposed to SSC medium for 5 to 10 seconds. Upon contact with SSC medium, the tail would begin to coil in the case of viable sperm or would remain unchanged in the case of nonviable sperm. The spermatozoon would then be flushed into iso-osmotic medium and washed several times. The injection needle also was washed by repeated aspiration of fresh medium to ensure removal of the SSC solution. The SSC test, in this case, revealed 0% viability of the sperm evaluated. Given the fact that only 1 oocyte was acceptable for injection, we decided, after consulting with the couple, to proceed with ICSI using the available sperm sample. A sperm was selected and was moved into a droplet of 7.5% polyvinyl-pyrrolidone. The sperm was rinsed several times before proceeding into the oocyte droplet for injection.

Thirty minutes after ICSI, the injected oocyte was activated with calcium ionophore. The injected oocyte was incubated in G-Fert (Vitrolife, Englewood, Colo) medium containing 10 μg/mL calcium ionophore

Correspondence to: Dr Ali Ahmady, University Fertility Centre, 23550 Hawthorne Blvd, Suite 210, Torrance, CA 90505 (e-mail: ali_ahmady@yahoo.com).

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A23187 (stock solution 10 mg/mL in dimethyl sulfoxide stored at −20C; Sigma Chemical Co, St Louis, Mo) for 10 minutes. The oocyte was then washed a few times in G-Fert medium prior to culturing in G1 medium. Fertilization was checked around 18 hours after injection. One oocyte was fertilized, and the zygote reached the 8-cells stage with excellent quality on day 3. The resulting embryo was transferred aided by ultrasound guidance. The patient conceived and underwent an uneventful pregnancy. She delivered a healthy baby girl at term.

### Discussion

It is known that oocyte injection of previously immotile sperm results in either poor or no fertilization (Dozertsev et al, 1995; Hoshi et al, 1995; Nijs et al, 1996). Poor fertilization could be due to inability of injected sperm to trigger the activation process of the oocyte to start fertilization. It is believed that live and intact sperm triggers calcium by introducing a soluble factor into the egg cytoplasm to initiate the activation process (Dale et al, 1985). We have already demonstrated that the key point in fertilization of nonviable sperm is activation of the injected oocytes. When the mouse and hamster oocytes were activated by injection of cytosolic sperm factor, the same fertilization rate, equivalent to injection with live intact sperm, was achieved (Ahmadi and Ng, 1999). In this report, we activated the injected human oocyte with calcium ionophore after injection of frozen-thawed nonviable testicular sperm. To our knowledge this is the first clinical report of pregnancy and delivery following transfer of embryo resulting from injection of a nonviable sperm and oocyte activation. It has been shown that calcium ionophore is effective to initiate the process of fertilization in failed cases after ICSI (Heindryckx et al, 2005). Successful pregnancy and delivery have been reported as a result of activation of oocyte injected with globozoospermic sample (Kim et al, 2001). We believe that by achieving fertilization following activation of the oocytes injected with nonviable sperm, we are opening a window of hope for similar patients so that they can have their own offspring.

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