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ASSISTED REPRODUCTION TECHNOLOGIES

Case report: live birth following ICSI with non-vital frozen-thawed testicular sperm and oocyte activation with calcium ionophore

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Introduction

The implementation of intracytoplasmic sperm injection (ICSI) in 1992 gave women whose male partners were diagnosed with severe male factor infertility the chance of having a baby using their partner's own ejaculated [1] or testicular spermatozoa [2]. Despite reported fertilization rates of approximately 70%, fertilization is dramatically reduced in some cases and does not even occur in 1–3% of ICSI cycles. Most cases of fertilization failure following ICSI can be traced back to a lack of oocyte activation [3], while injection of ejaculated immotile sperm results in either poor or no fertilization [4, 5]. The reason for this lack of oocyte activation may be a deficient cytosolic sperm-associated oocyte activating factor (SAOAF) resulting in a partial or complete inability of the sperm to activate the oocyte [4] or to an inability of the oocyte to decondense the sperm [6].

In 1997, oocyte activation with calcium ionophore was tested in a clinical trial that reported the delivery of a healthy baby [7]. In the same year, oocyte activation with calcium ionophore was performed using injected spermatids [8]. There have been several reports of successful oocyte activation by calcium ionophore treatment in patients with repeated failure of fertilisation using ICSI [9–11] or after ICSI using round-headed spermatozoa [10].

Capsule This is one of the first reports on the birth of a healthy baby following ICSI with frozen testicular spermatozoa showing no signs of vitality. In order to compensate reduced or missing sperm function the use of calcium ionophore treatment has been effective in achieving a pregnancy.

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Case report

Patient history

A 37-year-old woman and her 39-year-old husband presented at our institute for a first consultation. The husband had had radiotherapy following testicular cancer at the pre-cancer stage. Sperm freezing was not recommended for reasons unknown to us. After the radiotherapy, both testes had to be ablated. At the urology where the husband was operated they, however, recommended freezing testicular tissue before the start of the X-ray therapy, and four ampoules were frozen at that hospital in 2001. The urologist reports in his medical record that the testicular tissue in three of the four ampoules had not been mixed with the cryoprotectant, but had been frozen dry, suggesting that these three ampoules could not be used.

Material and method

Stimulation

The female patient received 50 µg thyroxine to compensate a moderate hypothyreosis. After down-regulation with Decapeptyl 0.1 mg/day (Ferring Arzneimittel GmbH) in a

conventional long-protocol, controlled ovarian hyperstimulation was performed with 225 IU/day HMG (IBSA Institut Biochimique SA) for 9 days. Final oocyte maturation and the induction of ovulation were performed with 10,000 IU hCG (Ferring Arzneimittel GmbH) upon observation of three follicles between 20 and 22 mm, four follicles between 15 and 17 mm and three follicles of about 12 mm.

Thirty-six hours after hCG injection, oocyte retrieval took place using transvaginal ultrasound guided follicle aspiration. Nine cumulus-oophorus complexes were collected, all of which were in metaphase II (MII).

Thawing of cryopreserved testicular biopsy sample

After thawing of the four ampoules no tubuli were observed in the tissue that had not been dissolved in the cryoprotective solution. No sperm was found either in the wet preparation or after discontinuous density gradient centrifugation. About 20 sperm were retrieved from the fourth ampoule after preparation. All sperm were immotile and had no flexible tail [13]. Treatment with pentoxyphylline did not result in motility.

Altogether, three biologists performed an evaluation during a 5-hour period. We considered oocyte activation with calcium ionophore as available data so far show benefits and no adverse effects and because this cycle was the only chance for the couple to conceive with the husband's sperm. We kept the remaining sperm for overnight incubation in order to confirm viability, however, the few sperm that had not been injected were still immotile and showed no viability according to the modified HOS test [14] as they did not show any coiling or swelling of the tail.

ICSI and oocyte activation with calcium ionophore

Three and a half hours after oocyte pick-up, enzymatic denudation of the cumulus cells using 40 IU/ml hyaluronidase (LifeGlobal) was performed. Thirty minutes after denudation, ICSI was performed according to Vanderzwalmen et al. [15]. Only immotile sperm presenting with no flexible tail were found and injected into 9 MII oocytes. Oocyte activation was performed as described by Heindryckx [16] with ionomycin (SIGMA). A 10 mM stock solution in DMSO (SIGMA, cell culture tested) was thawed and diluted twice in culture medium (LifeGlobal) in order to obtain a final concentration of 10 μM ionomycin. Thirty min after ICSI, injected oocytes were incubated in the ionomycin solution for 10 min. After thorough rinsing with culture medium the oocytes were kept in culture medium (LifeGlobal) for a further 20 min before incubation in the ionomycin solution was repeated for another 10 min. After a final washing the fertilized oocytes were further cultured in the one-step culture medium (Global; LifeGlobal) in 800 μl group culture in Nunc-4-Well dishes at 37°C and 6% CO2 in humidified conditions.

Fertilization was checked 18 h after ICSI. Six oocytes fertilized normally with two pronuclei and two polar bodies. On day 3, embryos were checked for number, size and grade of fragmentation. Four out of the six fertilized oocytes had cleaved and were transferred into a Nunc-4-Well dish containing fresh culture medium. Embryo transfer was performed on day 5. One embryo developed to a grade 4BB blastocyst [17] and was transferred using a Wallace transfer catheter.

Results

The patient delivered a healthy girl (3,470 g; 51 cm) by Caesarean section in pregnancy week 39.

Discussion

We report the birth of a healthy baby following ICSI with frozen testicular spermatozoa showing no signs of vitality.

According to Nagy et al. [18], lower rates of fertilization are associated with immotile sperm. In their study, Nijs et al. [5] obtained fertilization with immotile ejaculated and immotile testicular spermatozoa however, ongoing pregnancies only occurred with immotile testicular spermatozoa. Several studies have used the hypo-osmotic swelling test (HOS) to identify immotile sperm that are still viable [19].

Immotile testicular sperm can regain motility after several hours of in vitro culture [20]. De Oliveira et al. [13] reported a normal pregnancy after ICSI using immotile testicular sperm where viability was assessed using the mechanical touch technique to observe tail flexibility. It was suggested that the lower fertilization rates seen with immotile sperm may be due to the use of at least some nonviable sperm. In their retrospective study, De Oliveira et al. identified no significant differences between rates of fertilization, implantation and pregnancy with frozen and fresh testicular sperm of normal morphology and after injection of motile or immotile sperm selected mechanically.

In our case, the immotile sperm had no flexible tail and did not react to pentoxyphylline. We therefore postulated that they were not vital. We carried out a modified HOS test [14] and an overnight culture to confirm non-viability.

Even though severe male factor infertility can be overcome by ICSI, fertilization rates remains about 70%. More than 80% of unfertilized oocytes contain a spermatozoon [21]. Failed fertilization in these oocytes is caused either by the inability of the sperm to activate the oocyte or by an inability of the oocyte to decondense the sperm [6].

Oocyte activation is induced by a single rise in intracellular Ca^{2+} concentration when the sperm fuses with the oocyte membrane (trigger). A second phase with repeated calcium oscillations for 3–4 h (oscillator) is essential for complete oocyte activation [22, 23] and is induced by the release of a soluble sperm factor (SAOAF) into the cytoplasm of the oocyte. Vital and intact sperm can provide this soluble factor [24].

When ICSI is performed an increase in Ca^{2+} is artificially induced by the injection procedure approximately 30 min after ICSI. However, this is insufficient to fully activate the oocyte [22]. Sperm de-membranisation is necessary to facilitate the liberation of the cytosolic sperm factor responsible for the oscillator function [24, 25]. This can be achieved by immobilisation of the tail and the destabilization of the sperm membrane, resulting in the release of the cytosolic soluble sperm factor [15].

Many different methods to artificially induce oocyte activation have been reported [26], such as chemical activation by calcium ionophore with and without puromycin, electrical stimulation, strontium treatment and a modified ICSI technique. Chemical activation is the most commonly used and reported technique for stimulating oocytes and was first proposed for handling globozoospermia [10]. Reduced or missing sperm function can be compensated using a calcium ionophore. A spermatozoon may not be able to induce Ca^{2+} oscillations if it undergoes premature chromatin condensation or fails to decondensate at the proper time [27].

Combining calcium ionophore treatment with ICSI has been effective in achieving higher rates of fertilization, implantation, and pregnancy [9–12].

We decided to use calcium ionophore to activate the injected oocytes because this method has been applied in several studies with no evidence of toxicity or anomalies in the offspring.

Ahmady et al. [28] show that the key point in fertilization with nonviable sperm is the activation of the injected oocytes. Their case report presents a successful pregnancy and delivery following ICSI of frozen–thawed nonviable testicular spermatozoa after induction of oocyte activation with a calcium ionophore [29]. In 1997, Rybouchkin et al. [7] tested calcium ionophore treatment in a clinical trial, and a healthy baby was delivered.

In their study, Nasr-Esfahani et al. [30] applied artificial oocyte activation to a large number of patients with no adverse effects on the health of the infants to date. Furthermore, chromosomal analysis using FISH has shown a normal chromosomal status of those embryos derived through artificial oocyte activation [31]. However, the possible toxic or teratogenic effects have not been sufficiently analysed yet. Further animal studies will have to be undertaken before oocyte activation with calcium ionophore can be considered a standard procedure for treating fertilization failure.

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