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A Live Birth Following ICSI Using Frozen Immotile Sperm of Testicular Biopsy and Single Oocyte of Poor Responder Women

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Case Report

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Abstract

In this report, we present an unusual case of a couple who achieved a successful live birth by ICSI with a single oocyte injected in single frozen immotile sperm. The cumulus–oocyte–complex (COC) was retrieved at 36 hours post trigger, and was found to be at metaphase II, when a single injection of immotile sperm was performed from a Non–Obstructive Azoospermia testicle at approximately 39 hours after the trigger. At 19 hours post injection, the single oocyte was seen fertilized, developed to four–cell embryo on day 2, and transferred at 6–cell stage and graded well on day 3. The pregnancy yielded a positive β–hCG result. The scan performed at six weeks, revealed the presence of one gestation sac with a fetal heartbeat and a healthy female child was delivered after 9 months, with a birth weight of 2800 g and a birth length of 50 cm.

Keywords: In Vitro Fertilization (IVF); Cumulus–Oocyte–Complex (COC); Testicle; ICSI Time; Fertilization.

Introduction

In vitro fertilization (IVF) regimens of treatment, which are continuously being improved, have allowed the birth of over a million babies all over the world. One such improvement, which represents a major breakthrough in this area, is intracytoplasmic sperm injection (ICSI). Until 1992, most infertility failures originating from a severe male factor were untreatable. Micromanipulation techniques such as partial zona dissection [1–2] and sub zonal sperm injection [3–4], designed to overcome the poor performance of sperm cells, did not result in a substantial improvement of the rate of success of in vivo fertilization. However, ICSI, which was established by the team led by Professor Van Steirteghen at the Free University in Brussels, Belgium, and initially reported by Palermo et al. [4], has generated dramatic progress [5]. The ICSI procedure involves the injection of a single sperm cell intracytoplasmically into an egg.

The human ejaculate includes a very heterogeneous group of spermatozoa that vary widely in terms of their fertility potential and their ability to produce a high–quality embryo following ICSI [6]. Spermatozoa are highly specialized cells that acts vehicles that deliver the paternal genome to the oocytes. Although both sperm and oocyte genomes contribute equally to the developing embryo, it has been hypothesized that the extent of the sperm contribution to a successful live birth is a mere 10%–15% [7]. While this percentage appears to be limited, if an abnormal spermatozoa is used during ICSI, it could cause a significant impediment to embryo development. Therefore, it is safe to assume that ICSI outcomes and success rates will be dependent on sperm quality, among other factors. In support, embryo aneuploidies seen after assisted reproduction were significantly correlated with teratozoospermia [8]. The status of the paternal genome and sperm DNA fragmentation also appear to influence pregnancy rates and the risk of pregnancy loss following ICSI [9]. Similarly, it is important to note that poor in vivo reproductive outcomes including recurrent pregnancy loss have been associated with abnormal sperm parameters, including aneuploidies and poor DNA integrity [10].

The use of frozen–thawed ejaculated spermatozoa is well established in insemination cycles and IVF programs. Epididymal gametes from microsurgical epididymal sperm aspiration procedures also have been frozen, thawed, and used successfully for ICSI [11].

At present, data from animal studies point towards less favorable outcomes with frozen sperm utilization, implicating cryopreservation–induced damages to the cytoskeleton, DNA, and acrosome leading to adverse effects on spermatozoa's motility, viability, and ability to fuse with the oocyte. Assisted Reproductive Technology (ART) data, mostly focusing on severe male factor infertility diagnoses, suggest no major differences between in–vitro fertilization/ intracytoplasmic sperm injection (IVF/ICSI) cycles utilizing frozen over fresh sperm, often surgically extracted but this theoretically at lease cannot be applicable to TESA frozen sperm. It would be wise to mention the treatment of patient with oligospermia and Energy Level as important to achieve pregnancy [12].

Case Presentation

A married couple for 15 years visited the British–Syrian

Center at Al–Rasheed Hospital in Damascus (Syria) for ICSI on 4th April 2021, trying to conceive for 10 years, history of previous 3 failed attempts of ICSI during 15 years of marriage without any pregnancy, bearing in mind that the husband's age is 42 years diagnostic of non–obstructive azoosperermiac with FSH 19. wife aged 39 years, a testicular biopsy TESA (testicular sperm aspiration) was performed 1 month prior to ICSI attempt, result showed presence of a very few immotile sperms in the left testicle upper region, we discussed the chance of frozen immotile sperm sample and agreed to freeze the sample for future use if needed. Since the regulation of local health authority documented egg donation as a forbidden approach.The couple underwent a ICSI attempt The initial consultation showed wife with low antral follicle counts (AFC) and AMH 0.4. with FSH: 15 mIU/mL, E2: 41 pg/mL while TSH, FT4, Prolactin within normal range.

150 IU daily of human gonadotropins (Menopur, Ferring, Germany) were administrated for twelve days (total gonadotrophin dose of 1800 IU) using Antagonist flexible protocol (Ctrorelix acitate "Cetrotide" Serono) with Ovitrelle 250microgram/0.5ml, Merck, as trigger ovulation and Oocyte maturation. One dominant follicle was seen on the right ovary, whereas no follicles were seen on the left ovary. triggered by using a single injection of Ovitrelle SC injected at 10pm, 36 hours before egg collection, Endometrium measured 9.8 mm . Transvaginal ultrasound–guided oocyte retrieval was carried out; only one COC was retrieved during the oocyte pick up.

OOCYTE retrieval, Retrieval of sperm from testicular tissue, ICSI embryo culture and transfer

The cumulus–oocyte–complex (COC) was isolated from follicular fluid and then rinsed in 1.0 ml G–MOPS™ plus medium (Vitrolife, Göteborg, Sweden). Following the oocyte pick–up, the oocyte was transferred to a 1.0 ml equilibrated G–IVF™ medium (Vitrolife) at 37°C, and 6% CO2, in a Labotect incubator (Labotect, Lab C201, Germany) until the time of ICSI.

TESA was performed under local anesthesia. A 16–gauge clear angiocatheter needle (1–1/400 CATHLON I.V Catheter; Smiths Medical International Ltd, Rossendale, UK) is directed through the scrotal skin into the testis. The needle is withdrawn and the angiocatheter is kept in place [13]. A 10 mL syringe containing 1–2 mL of sperm buffer is attached to the angiocatheter. Negative pressure is created and the angiocatheter is gently withdrawn and then pushed back into the testis until testicular tissue appears in the syringe. At this point, the angiocatheter is withdrawn completely while maintaining negative pressure and the remaining tissue is pulled using a smooth forceps. The testicular tissue is then expelled into a sterile dish. The specimen is immediately dissected and then examined under the microscope to confirm the presence of spermatozoa. The same technique is repeated (up to three aspirations per side), Immediately after withdrawing the angiocatheter needle, gentle pressure is applied to the puncture site for 2–3 min, so as to minimize the risk of bleeding. no sperm were found, the decision was taken to thaw the frozen sample [14]. For previous frozen sample in which spermatozoa were identified in the wet preparation immediately after the TESE procedure, the biopsy specimen was frozen in one ampoule for subsequent use in therapeutic ICSI cycle. Use technique freeze and thaw short protocol of media sperm freeze solution (Vitrolife, Sweden).

Approximately 39 hours after trigger, the oocyte was treated with hyaluronidase (80 mIU/ml) for 40 seconds in order to remove the surrounding cumulus cells. At that time, the oocyte was found to be at the MII stage, with clear extrusion of the first polar body.

One immotile sperm out of normal morphology sperm sample was selected using the mechanical touch method under an inverted microscope (Nikon Eclipse Ti–S, Japan) from frozen sample, and microinjected with the use of electrohydraulic injectors (Narishige, Japan). The oocyte was kept still by using a holding micropipette at the 9 o'clock position, and the polar body was oriented at the 12 o'clock position. The injecting pipette was then gently advanced through the zona pellucida and oolemma, until the pipette was beyond the center of the oocyte; then, the sperm was gently deposited into the oocyte's cytoplasm. The oocyte was examined for the presence of two pronuclei, and successful fertilization was confirmed at approximately 19 hours after insemination.

On day 2, the embryo was a four–cell grade one, reaching six–cells grade one on day 3, when it was transferred to the uterine cavity (Figure 1). The embryo culture was completed adopting a sequential preequilibrated medium (Vitrolife G–series) as follows. Firstly, the fertilized oocyte was placed into a 20–microliter drop of G–1™ media, covered by light paraffin oil, sterile filtered (OVOIL–Culture Oil, Vitrolife, Sweden) [15]. On the morning of day 3, the embryo was transferred from the G–1™ micro droplet to a 20–microliter droplet of G–2™ medium, and kept in culture until 3 hours, when the embryo transfer was performed. We hereby report a compelling case in which an ongoing pregnancy with fetal heartbeat was obtained from the only oocyte collected after COS with immotile frozen sperm. The embryo replacement was completed under transabdominal ultrasound guidance using a soft transfer catheter (Wallace TM Classic, Cooper Surgical, USA). We started the luteal phase support with 90mg progesterone vaginal gel twice a day (Crinone 8%, Merck) on the evening of egg collection day and continued till the 8 ®gestational week, a tablet of baby Aspirin 75mg daily with folic acid one tablet a day was added.

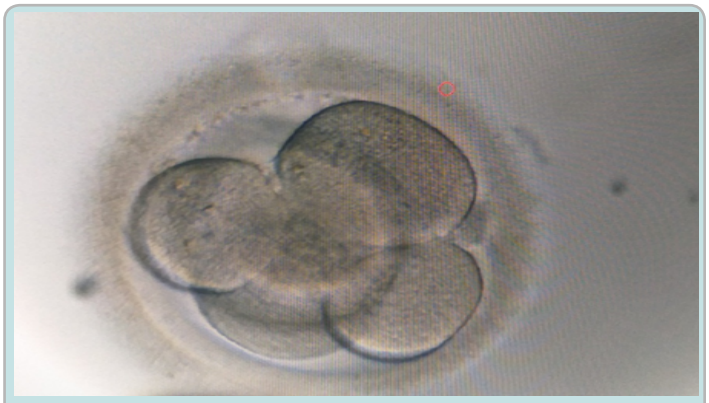

Figure 1: Embryo transferred on day 3.

Hormone Measurement

Serum progesterone and estradiol level was measured on the day of hCG administration. Sample was tested with a microparticle enzyme immunoassay (Axsym System, Advia Centaur; Sie– mens). The results progesterone and estradiol 0.9 ng/mL , 425 pg/ mL, respectively.

Pregnancy outcome

First ultrasound scan was performed six weeks later, which revealed the presence of one gestational sac with fetal heartbeat, the pregnancy was monitored until birth according to UK royal collage guidance. At 39 weeks caesarean section performed after long discussion with the couple as overdue, resulted a baby female with good Apgar weighing 2,9kg on 13th February 2022.

Discussion

The use of assisted reproduction technology (ART) to overcome infertility has increased steadily in the worldwide. ART generally includes treatments such as in vitro fertilization (IVF), gamete intrafallopian transfer and zygote intrafallopian transfer, with IVF accounting for approximately 99% of all ART procedures. Intracytoplasmic sperm injection (ICSI) involves the injection of a single spermatozoon directly into the cytoplasm of an oocyte. ICSI bypasses both the ZP barrier and sperm defects in the male gamete that compromise its ability to fertilize. The ability of ICSI to achieve higher fertilization and pregnancy rates regardless of sperm characteristics makes it the most powerful micromanipulation procedure yet for treating male factor infertility [16]. In fact, the therapeutic possibilities of ICSI range from cases in which, after sperm selection, the spermatozoa shows poor progressive motility, to its application in azoospermic men where spermatozoa are microsurgically retrieved from the epididymis and the testis [17]. Retrieval of a low number of oocytes represents a further indication for this procedure. IVF outcome dependents on multiple factors, including oocyte and sperm quality, maternal age, infertility cause, lifestyle factors, as well as laboratory conditions such as manipulation and embryo culture [18–19]. Several authors have reported that the waiting time of 2 or 4 hours between oocyte retrieval and insemination improves fertilization rate, embryo quality and pregnancy outcomes [20] and we applied this this rules.

Schoysman et al. [21], were the first to report fertilization and pregnancy after ICSI with sperm obtained by testicular sperm extraction (TESE). Even in cases of testicular failure, TESE can be used successfully [22]. Case reports of successful ICSI with frozen–thawed testicular motile sperm have been published [23–24], but there are few reports of success with immotile sperm [25]. Nagy et al . [26] stressed the role of sperm motility for successful outcome of ICSI, and in 1998 reported lower rates of fertilization associated with non-motile testicular sperm. However, the criteria for selection of viable immotile sperm were not reported. The mechanical touch technique used in our centre involves laterally pressing against the upper third of the immotile spermatozoon tail and the ICSI dish with the ICSI micropipette (Humagen), much as is routinely done to immobilize motile sperm, thus forcing the tail to one side: The micropipette is raised, and the response is observed. If the tail is flexible and recovers its original position, it is considered viable. Sperm rigidity and incapacity to recover the initial tail position is considered a sign of non-viability. The success and reliability of this technique depend largely on the expertise of the biologist performing the assessment. Found study [27], the reduced number of motile spermatozoa had declined the fertility ability and embryo quality during ICSI treatment of severe oligozoospermia [28]. The crucial finding was that the prospective clinical outcomes could be achieved as long as well selected good–quality embryos were transferred, in our study no chose was left, regardless of whatever severity of oligozoospermia. Whoever in our case we infesis to select immotile perm after thawing frozen immotile sperm sample could lead to a life birth since the sperm donation is not an option in our country as local health authority regulation, In contrast, transfer of lower quality embryos reduced the rates of pregnancy, implantation and live birth [29], indicated a lower clinical pregnancy rate when using non–motile sperm for ICSI, all study couples with female factor infertility and/or female age ≥39–year–old were excluded. Also in this study the poor responder lady included. Successful [30], use the laser method to select non–motile testis sperm in ICSI to obtain high quality embryos, while we used the mechanical touch method for sperm selection.

Health professionals providing care to infertility patients should be aware that it is possible to provide couples, like the one described here, with a real hope of biological parenthood. Equally important is to share such cases within the MAR community, which is often skeptical about the likelihood of one collected oocyte with a non–motile sperm to generate a viable embryo and pregnancy then fetal heartbeat observed and a successful live birth.

Conclusion

We report here a compelling case in which a 39 year old, poor responder, woman after COS was able to produce one follicle. Approximately 36 hours after trigger, one oocyte was collected and then fertilized by ICSI from one frozen immotile sperm obtained from a testicular biopsy with the selection of the sperm by mechanical touch method. Our case–report stimulates the discussion on the above aspects particularly in view of egg or sperm donation is not an option according to local authority. The case reported achieving successful pregnancy with multi–unfavorable female and male factors i.e. low AMH and poor responder, age factor and frozen immotile sperm sample.

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None.

Conflict of interest

Authors declare that there is no conflict of interest.

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