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Successful Application of the Sperm Tail Flexibility Test for Immotile but Viable Sperm from a Cryopreserved Retrograde Ejaculate

Khaliq Ahmad, Samuel Prien*, Lindsay Penrose, Bo Suh

Department of Obstetrics and Gynecology, Texas Tech University Health Sciences Center, Lubbock, Texas, USA
Email: khaliq.ahmad@ttuhsc.edu, *Samuel.prien@ttuhsc.edu, lindsay.penrose@ttuhsc.edu, boysuh@ttuhsc.edu

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Abstract

The use of intracytoplasmic sperm injection (ICSI) has become the mainstay of oocyte fertilization for assisted reproduction. However, the traditional technique requires a motile sperm population for sperm selection. Occasionally, the embryologist is faced with entirely nonmotile sperm populations with no alternative but to use nonmotile cells for injection. The following report describes a successful application of the sperm flexibility test for viable sperm selection from a non-motile population.

Keywords

ICSI, Retrograde, Sperm Tail Flexibility Test

1. Introduction

Intracytoplasmic sperm injection (ICSI) has become a preferred method of achieving successful fertilization of oocytes, especially in cases of male factor infertility in assisted reproductive techniques (ART) laboratory procedures. A generally accepted pre-requisite for ICSI is the selection of a single viable sperm typically indicated by motility from available sperm population. However, motile sperm may occasionally be absent to perform ICSI especially in treatment cycles involving surgically extracted testicular sperm or certain ejaculated specimens. To aid in selection, various techniques have been utilized to identify viable sperm from the immotile population. These include: (i) the hypo-osmotic swelling test; (ii) chemical substances such as pentoxifylline and theophylline for initiating tail movement; (iii) the sperm tail flexibility test (STFT); and (iv) laser assisted immotile sperm selection [1]-[3]. In this report, we describe a case of successful fertilization of oocytes by ICSI and subsequent embryo development using viable sperm iden-

tified through STFT from frozen-thawed immotile sperm population originating from retrograde ejaculation.

2. Case History

A couple was referred to our university-based center for fertility & reproductive surgery for infertility evaluation. The female partner was 34 years old, G2P2 with a history of dysmenorrhea and prior tubal ligation. She reported regular monthly cycles and expressed a desire to conceive with her current partner. Laboratory evaluation revealed anti-Mullerian hormone (AMH) value of 5.7 ng/ml and cycle day 3 follicle stimulating hormone (FSH) value of 9.5 mIU/ml, both values appropriate for her age and indicative of a favorable ovarian reserve.

Her male partner, aged 36, had a longstanding history of type 1 diabetes mellitus and was diagnosed with retrograde ejaculation, contributing to the couple's infertility.

The female patient underwent a treatment cycle of *in vitro* fertilization (IVF) with ICSI following controlled ovarian hyperstimulation that involved pituitary down-regulation using a combination of leuprolide acetate and oral contraceptive pills, followed by daily gonadotropic injections beginning on cycle day 3, in conjunction with a reduced dose of leuprolide acetate. Follicular development was monitored through serial estradiol measurements and transvaginal ultrasound examinations.

A total of 2700 IU of recombinant FSH (Gonal F, Serono Pharmaceutical) was administered prior to triggering ovulation on day 10 with 10,000 IU of human chorionic gonadotropin (hCG). The endometrial thickness measured 10.00 mm by transvaginal ultrasound on trigger day. A total of 18 oocytes were retrieved via transvaginal ultrasound guided follicular aspiration approximately 34 hours after hCG trigger.

Spermatozoa for ICSI were obtained from a previously cryopreserved sperm sample recovered from a retrograde ejaculation. The specimen was thawed by immersing the vial at 37˚C for 5 minutes and washed by a simple wash procedure using Hepes-HTF medium (*in vitro* care) supplemented with 10% synthetic serum substitute (SSS, Fuji Film/Irvine Scientific) and concentrated to 0.25 ml volume.

The final washed specimen had a sperm concentration of 0.02 million/ml and 0% motility. Sperm selection for ICSI was determined by sperm tail flexibility test (STFT). Briefly, 5 µl of washed sperm suspension was added to a 5 µl drop of Hepes-HTF medium + 10% SSS on ICSI dish and overlayed with mineral oil.

To identify viable sperm within a completely non-motile population, STFT was employed. For this purpose, the sperm tail was gently manipulated with ICSI needle. If the tail moved independently in an up-and-down motion without concurrent movement of head, the sperm was considered to be viable [4] and was selected for ICSI procedure. Conversely, if the head moved in conjunction with the tail, sperm was deemed non-viable and excluded from use.

For IVF, 14 metaphase-II stage oocytes were injected with STFT selected sperm. Fertilization was confirmed in 9 oocytes (fertilization rate of 64.28%) as indicated

by the presence of 2 pronuclei approximately 18 hours post-ICSI. The resulting zygotes underwent extended embryo culture in Global medium (Life Global Group) supplemented with 10% SSS for up to 6 days at 37˚C under a reduced O_2 environment (5% O_2 + 6% CO_2 + balance nitrogen).

Seven good-quality, transferable blastocysts developed by day 6 in culture (blastocyst development rate of 77.77%). On day 5, 2 high grade blastocysts were transferred back to the uterus of female patient (Gardner's scale grades 4AA and 4AB) and remaining 5 blastocysts were cryopreserved for future use. Luteal phase support was provided via daily intramuscular injections of progesterone in oil (50 mg/day). Quantitative βhCG testing performed 10 days post-transfer revealed a negative pregnancy result. The patient has not yet returned to the clinic to attempt conception by using her cryopreserved embryos.

3. Discussion

While selection of a viable sperm indicated by motility has been the gold standard for ICSI, selection of viable sperm from non-motile sperm population remains challenging. The most commonly used technique for assessing viability in non-motile spermatozoa is the hypo-osmotic swelling (HOS) test [5]. Exposure to hypo-osmotic solution causes the sperm tail with intact membranes to swell or curve, indicating viability [6] [7] and such sperm may then be used to perform ICSI. However, a notable drawback of HOS selected sperm is the difficulty encountered during aspiration of sperm into ICSI needle due to swollen or curved tail. To mitigate this, HOS selected sperm must be incubated in normo-osmotic solution for 30 - 60 minutes prior to use [8]-[10]. This additional incubation step introduces complexity to ICSI workflow and may prolong the already demanding schedule of ART laboratory personnel.

In contrast, the sperm tail flexibility test (STFT) used in this case report offers a simpler alternative. STFT involves a gentle mechanical touch of the sperm tail with the ICSI needle, without compromising sperm integrity or requiring exposure to specialized solutions. Sperm are handled in HEPES-buffered HTF medium, routinely used in ART laboratories, and no normo-osmotic incubation is necessary, streamlining the ICSI process. In our experience, viable sperm identified via STFT from a frozen-thawed, non-motile population exhibited no tail curling or swelling, and aspiration into the ICSI needle was smooth and efficient.

Another approach for selecting viable sperm for ICSI involves chemical activation of tail movement by using chemical agents such as pentoxifylline and theophylline. These compounds induce sperm motility by inhibiting phosphodiesterase activity, thereby increasing intracellular cyclic adenosine monophosphate (cAMP) levels [11] [12]. Incubation with pentoxifylline has been reported to significantly improve sperm motility and forward progression in cases of oligozoospermia and asthenozoospermia and helped to overcome fertilization failure in previous conventional inseminated IVF treatment cycles [11]. However, safety concerns have been raised regarding the use of these chemicals in routine ART

lab practice, particularly due to their potential adverse effects on human sperm function and embryo development. In contrast, viable sperm selection from immotile population by use of STFT does not seem to pose any chemical threat to sperm function and subsequently developing embryos. Further, STFT selected sperm can immediately be used for ICSI without need for incubation in wash solutions and delays, thereby minimizing disruption to ART laboratory's workflow.

Laser assisted identification of viable but immotile sperm has been reported [2] [3] [13]. This technique involves delivering a single low-intensity laser pulse shot directed at the tip of sperm tail. In viable but immotile sperm, this stimulation causes a coiling or curling of the tail. Conversely, absence of such a response indicates non-viability. Use of laser selected sperm from asthenozoospermic samples resulted in significantly higher fertilization and cleavage rates after ICSI as compared to those resulting from randomly selected sperm [14]. However, this technique requires substantial expertise in laser operation and access to specialized and costly equipment. In contrast, STFT offers a simpler and more accessible method of identifying viable sperm. STFT relies upon mechanical touch with ICSI needle, a skill readily mastered by technologists experienced in ICSI. Importantly, STFT utilizes standard equipment already present in ART laboratories, eliminating the need for additional investment in laser technology.

In the present report, viable sperm selection from non-motile frozen-thawed specimen originating from retrograde ejaculation was successfully accomplished by using the STFT technique to fertilize oocytes in an IVF/ICSI treatment cycle. ICSI with selected sperm yielded a fertilization rate of 64.28% compared to our laboratory's benchmark rate of 70% for ICSI. Notably, the subsequent blastocyst development rate was substantially higher at 77.77% compared to our laboratory's benchmark rate of 50%.

Due to its simplicity and cost-effective nature requiring no special equipment, rinsing media or incubation prior to ICSI procedure, the STFT can be readily integrated into routine ART laboratory workflow. This technique is particularly advantageous in IVF treatment cases involving ejaculated asthenozoospermic specimen, non-motile sperm from testicular extraction (fresh or frozen) or retrograde ejaculated specimen (fresh or frozen).

While the initial transfer did not result in pregnancy, the couple has a large cohort of embryos left for future attempts. The incidence of complete lack of motility is uncommon in any single laboratory setting. However, it might be worthwhile to conduct a research survey of a cross-section of programs to determine how such cases are dealt with and their outcomes.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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