



Case Report: Birth of Healthy Infants After ICSI with Pentoxifylline Treated TESE Sperms in Patient with Advanced Maternal Age

Talha Paracha*

Sr. Embryologist, Msc, Biotechnology, Associate Member Royal College of Pathology UK, LIFE IVF Multan, Pakistan

*Corresponding Author: Talha Paracha, Sr. Embryologist, Msc, Biotechnology, Associate Member Royal College of Pathology UK, LIFE IVF Multan, Pakistan.

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Abstract

Intracytoplasmic Sperm injection has been a method of choice in patients suffering from Azoospermia, where sperm retrieved up on biopsy are usually immotile and to perform insemination, effectively selection of viable spermatozoa is prerequisite to ascertain the outcome. We utilized low concentrations of Pentoxifylline in Non-Obstructive Azoospermic patient that underwent TESE (Testicular Sperm Extraction) in our IVF programme, resulted in healthy singleton up on Fresh Embryo Transfer and Fraternal healthy babies up on Frozen Embryo transfer.

Keywords: TESE (Testicular Sperm Extraction); IVF Programme

Introduction

Non-Obstructive Azoospermia is the most severe form of male factor infertility, which is due to defective spermatogenesis, because of genetic abnormalities or idiopathic causes and accounting for about 15% of male infertility [1]. ICSI could not have been performed without differentiation of viable from non-viable sperms therefore pentoxifylline was utilized.

Case Report

A 36 year old female patient presented with primary subfertility for 2 to 3 years and was seeking treatment at LIFE, IVF Multan. Female patient was found with no complications up on evaluation. Male patient aged 37 was declared Azoospermia upon semen analysis and was diagnosed Secondary Non Obstructive Azoospermia up on further investigations. His bio markers were before treatment FSH: 14.3 mIU/ml, LH 7.9 mIU/ml, Prolactin 12.4 ng/ml, Testosterone: 534 ng/ml. Male patient was treated with gonadotropins which improved Serum Testosterone to 784.3 ng/ml and LH: 8.6 mIU/ml.

Short/ Flare Simulation was used for ovarian stimulation. Ultrasound guided Trans Vaginal Oocyte retrieval was performed 34-36 hours later [2].

Testicular sperm preparation

On day of Oocyte retrieval no sperm was observed in fresh TESA sample, so Open Testicular Biopsy was performed.

The extracted testicular tissue was placed in petridish with GMOPS Plus Media (Vitro Life, Sweden) and tissue was minced with 2 sterile blades into suspension and transferred to conical bottom test tube. These tubes were then centrifuged at 400 g for 5 minutes, and the suspension was discarded. The pellet was suspended with GMOPS-Plus medium (VitroLife, Sweden). The specimen was then incubated at 37°C in warming incubator for use in ICSI [1].

ICSI and sperm activation

5 ul droplet was made in ICSI dish of biopsy sample followed by 10ul droplet of PVP adjacent and surrounding GMOPS plus (Vitro-life, Sweden) droplets for insemination. When no motile spermatozoa was observed or when average mean time to find spermatozoa was greater than 25 minutes/individual spermatozoa. An equal volume of (5ul) of 3 mmol/Lit of Pentoxifylline (Sigma Aldrich) was made with GMOPS Plus simultaneously and was added to droplet directly through Mineral oil there by giving a concentration of 1.5mMol/Lit. After incubation of 10 minutes, motile spermatozoa (Showing Vibratory motion) were aspirated via ICSI injection pipette, rinsed in a droplet of culture media before being transferred to PVP for injection [3].

Total of 11 oocytes were obtained 8 oocytes were at M2 stage and were inseminated, fertilization was observed 18 hours of insemination. Inseminated Oocytes were cultured over night at 6% CO₂, 5% O₂ and 89% Nitrogen under humidified conditions to observe fertilization at 18th hour post insemination. Total 6 oocytes were fertilized and 4 Blastocyst were obtained.

Fresh and frozen embryo transfer

For Fresh Embryo transfer progesterone support was started from day of Oocyte retrieval and embryo transfer as on HCG+7th day of priming. For Frozen Embryo transfer HRT cycle was selected for preparation of Endometrial lining [4].

Embryo Transfer was performed using Cook Medical Embryo Transfer Catheter (Ref No 6019) Pregnancy was proved by quantitative determination of B.Hcg in serum, and implantation by Ultra - Sonographic Evidence of Gestation sac with heart beat 4 weeks after Embryo Transfer.

Results

A healthy singleton was delivered after Fresh Embryo transfer and up on Frozen Embryo transfer Fraternal twin babies were delivered normal in this patient.

Discussion

Sperms obtained from Testicular biopsied tissues are immotile mainly because final maturation and storage takes place in Epidymis to induce motility [5]. Currently, we use the Pentoxifylline to activate the motility of sperm in TESE-ICSI procedure. The Pentoxifylline acts as an inhibitor to phosphodiesterase which consequently results in cAMP that leads to increase in PK-A which in turn results in sperm motility [6].

Pentoxifylline helps to distinguish vital from non vital spermatozoa by activating motility in spermatozoa rapidly which is followed with an immediate ICSI there by which a rapid single step is generated which enables to differentiate between vital non vital spermatozoa and decrease time taken for successful injection and optimize results.

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