Acta Scientific Women's Health (ISSN: 2582-3205)

Volume 6 Issue 3 March 2024

Case Study

# Successful Pregnancy After ICSI with TESE Sample in Serodiscordant Couple with Male Partner Sero Positive for HCV After Utilizing Modified Washing and Chemical Activation of Sperms Simultaneously

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**Received:** February 02, 2024

**Published:** February 28, 2024

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## Abstract

An infertile couple seeking fertility treatment from LIFE IVF Multan, Pakistan, where male was diagnosed with HCV positive and NOA. Female partner had her Fallopian tubes blocked while rest of the parameters were in normal ranges .Couple was advised for Testicular Biopsy and to undergo ICSI cycle .Only hindrance was blood contaminating testicular tissue with viral particles so an modified washing technique was adopted mentioned elsewhere(2), claiming to reduce viral load in biopsied sample which was followed by vitrification of sample to further use in ICSI cycle .On day of procedure biopsied sample was thawed and observed for sperm motility which on absence was subjected to chemical activation .Couple was briefly informed on possible adverse outcomes low fertilization, Poor quality embryo, risk of cross infection and lack of adequate data on methodology adopted. The methodology adapted resulted in successful pregnancy, Mother was investigated for Anti HCV Abs on 1st trimester and after successful delivery of healthy baby ,both mother and infant was investigated again for Anti HCV Abs and both were found negative .This gives a preliminary proof for safety of the procedure adopted.

**Keywords:** Pregnancy; ICSI; TESE; Serodiscordant; HCV; Sperms

## Introduction

Non Obstructive Azoospermia is considered as most severe form of male infertility and serodiscordat Couples having male partner positive for HCV OR HIV with NOA condition are usually considered at higher risk for cross infection as blood in testicular tissue samples due to open biopsy surgical procedure can cause contamination of virus in TESE samples and risk of transmission (Cross Infection) exists more in comparison to ejaculated samples washed after Gradient centrifugation. Some Laboratories prudentially denied any treatment to such couples: others have admitted the couples to their IVF Programm,s but ,unfortunately have transmitted HCV RNA from serum positive to serum negative women [7].

It is postulated that not only in Density Gradients but also in simple culture medium ,sample centrifugation may produce an effective separation of spermatozoa from viral particles due to their different mass ,leaving the virus in the supernatant and spermatozoa in the pellet [1].

Here we utilized a protocol for testicular sperms washing mentioned previously with confident result to remove as maximum possible viral load from a testicular tissue [2] in conjunction with Chemical activation of sperms which in most of the cases with NOA and frozen thaw don't have the motility at all.

## Case Report

A couple with primary Sub-fertility for 2 to 3 years was seeking treatment at LIFE IVF, Multan, Pakistan with female age 21 years and male with 32 years .Female was diagnosed with tubal blockage ,rest all biochemical markers, ultrasounds/HSG reports ,and cycle were regular and normal, whereas male was diagnosed with NOA alongside Hepatitis C.

Couple was counseled in brief for ICSI, Testicular Biopsy for retrieval of sperms, Cryodamage to TESE sample and possible chances of low fertilization and viral transmission (cross infection).

Testicular sperm preparation and modified washing

The pieces of testicular tissues were placed in petri dishes with GMOPLS plus (Vitrolife) medium and washed to remove the blood, after washing biopsy sample was transferred to another dish containing same medium and seminiferous tubules were dilacerated utilizing sterile needle s. A drop of 20 to 30 ul was observed under inverted Microscope to confirm the presence of sperms.

The suspension was then centrifuged at 300x g for 20 minutes on 1 ml Layer of 45% Gradient. After centrifugation the sperm pel-

let was carefully collected and re-suspended in 5 ml of GMOPS Plus medium .It was then centrifuged for 10 minutes at 600 x g .This washing step was reiterated once. After 2 washings, the sperm pellet was re-suspended in 0.5 -1.5 ml of the same medium and sperm number and motility was evaluated again [2].

This suspension was then vitrified using Sperm Freeze medium (Fertipro) according to manufacturer's instruction and protocol.

### Sperms activation

As sperms are usually immotile in most of the cases of NOA and vitrification has also some impact on motility of sperms motility was not observed in post thawed sample, therefore chemical activation via Pentoxifylline was necessary.

Pentoxifylline solution was added to droplets to give a final concentration of 1.5 mmol/lit. After incubation for 10 minutes Embryologist pick the motile sperms and transferred to washing droplets using Micro needle, the sperms were then washed and immobilized in PVP before transferred to oocyte placed drop for ICSI [3]. Ovarian Stimulation and Oocyte Retrieval.

Short/Flare protocol was used for Ovarian Hyper stimulation Ultrasound guided trans vaginal oocyte retrieval was performed 34-36 hours after HCG trigger [4].

A total of 18 Oocytes were retrieved ,14 were at mature M(II) stage which were then inseminated .Among 14 oocytes 09 oocytes were fertilized and 6 among them reached to Blastocyst stage which were then vitrified using Kitazato Vitrification kit according to manufacturer's protocols.

### Frozen embryo transfer

For Frozen Embryo Transfer HRT cycle was selected for preparation of Endometrium Lining [5]. On day of Embryo Transfer 2 Expanded Blastocyst of grade 4AA were transferred. 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 songraphic evidence of Gestation Sac with heart beat 4 weeks after Embryo transfer.

A healthy singleton baby was delivered, both mother and child was investigated for antibody titer after 3 months of delivery and were found negative.

## Discussion

Non Obstructive Azoospermia is considered as most severe form of male infertility where sperm are retrieved by Open testicular biopsy which is often associated with the presence of blood due to surgical procedure and in serodiscordant couples where male are HCV or HIV positive is a potential source of cross infection/ transmission to sero-negative partner. ART produces more responsibilities :of the physician towards the conceptus of the non-infected partner ,of the other couples and of the employer towards the IVF Laboratory staff .A general agreement on this issue is lacking ,and different IVF Laboratories adopt different behaviors [1].

HCV RNA was found below detection levels after gradient centrifugation of ejaculated specimen followed by swim up and washing of the sperms [6,7].

Based on this knowledge here in this case we adopted a unique washing method for processing of testicular biopsy sperm [2] which claims to be producing final specimen devoid or with vrial load beyond detection level for seropositive males, By adopting this procedure, it resulted in a successful pregnancy full term with normal baby delivered and mother and infant were further investigated for presence of Anti HCV Abs which found negative were thereby giving a preliminary proof of the safety and efficacy of the procedure.

## Conclusion

It is mandatory in serodiscordant couples where male are positive for HCV /HIV that sperms must be free from any infection for safe IVF procedure and this new method of processing sample helps in this regards.

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