

## Case Report

# The First Report of Successfully Pregnancy after ICSI with Combined DGC/Zeta Sperm Selection Procedure in a Couple with Eleven Repeated Fail IVF/ICSI Cycles

Mohammad Reza Deemeh, M.Sc.<sup>1,2</sup>, Marziyeh Tavalaei, M.Sc.<sup>2</sup>, Seyed Mahdi Ahmadi, M.D.<sup>1</sup>, Seyed Asadolah Kalantari, M.D.<sup>1</sup>, Seyed Vali Alavi Nasab, M.D.<sup>1</sup>, Mohammad Hassan Najafi, M.D.<sup>1</sup>, Mohammad Hossein Nasr Esfahani, Ph.D.<sup>1,2\*</sup>

1. Isfahan Fertility and Infertility Center, Isfahan, Iran

2. Reproduction and Development Department, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran

### Abstract

This is the first report implementing combined density gradient centrifugation/Zeta (DGC/Zeta) sperm selection procedure for a couple with eleven previous intra cytoplasmic sperm injection/*in vitro* fertilization (ICSI/IVF) failure cycles. Semen analysis was carried out according to World Health Organization criteria. Protamine deficiency, DNA fragmentation and morphology were assessed by chromomycin A3 (CMA3), TUNEL assay and papanicolaou staining, respectively. Patient was counseled regarding DGC/Zeta sperm preparation procedure. 10 oocytes were injected with combined DGC/Zeta sperm preparation which resulted in 90% fertilization rate and eight embryos with good quality. Three embryos were transferred on day three. Singleton pregnancy and healthy girl baby delivered with cesarean section. Result of this case report opens the horizon for further evaluation Zeta of sperm selection procedure for couples with repeated ICSI/IVF failure.

**Keywords:** Intracytoplasmic Sperm Injections, Density Gradient Centrifugation, Fertilization, Pregnancy

### Introduction

By introduction of intracytoplasmic sperm injection (ICSI), to the field of infertility, new hope was given to infertile couple, to have a child with their own gametes (1). In this technique, the barriers in natural fertilization are circumvented by visual selection criteria based on motility and morphology. Since sperm genomic integrity is of fundamental importance to fertilization and embryo development and visual selection can account for this factor (2), therefore, insemination of sperm with chromatin anomalies and fragmented DNA can result in failed fertilization or failed embryo development post fertilization and increased rates of miscarriage and diseases in the offspring, including childhood cancer (3, 4).

One approach to obtaining information for the selected sperm is to evaluate or assess the sperm normality and DNA integrity of the sperm fraction selected for injection. In addition, recent study suggests that considerable percentage of sperm tagged as normal morphology according to strict criteria is apoptotic or TUNEL positive (5).

In addition, considering the fact that there is a

strong correlation between semen parameters and percentage of sperm with damaged DNA (4), there is a high chance for inappropriate sperm to be inseminated in cases with extremely poor semen samples. So there is very reason for concern regarding insemination of sperm with abnormal chromatin structure and DNA fragmentation. Therefore, new sperm selection procedures have been developed based on sperm molecular or function characteristics for ICSI, especially for sever male factors or those with repeated failure. These methods include, sperm magnetic cell sorter to select non apoptotic spermatozoa (6), hyaluronic acid binding that based on binding of sperm to solid HA via sperm HA receptors (7, 8), electrophoretically isolated spermatozoa and Zeta methods based on sperm membrane charge (9, 10).

Chan et al 2006 initially showed that the Zeta method is a simple and reliable method to select sperm with normal chromatin status (10). In addition, further studies reveal that highest quality spermatozoa in the ejaculate are the most electronegative (11, 12). Our previous study further verified that sperm selected based on sperm Zeta po-

Received: 19 Jan 2010, Accepted: 11 Mar 2010

\* Corresponding Address: P.O.Box: 19395-4644, Reproduction and Development Department, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran

Email: mh.nasr-esfahani@royaninstitute.org



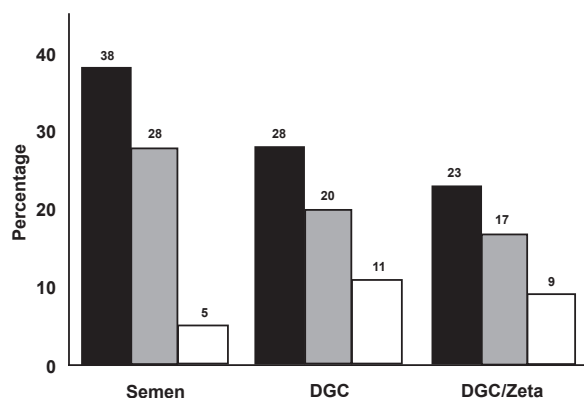
Royan Institute  
International Journal of Fertility and Sterility  
Vol 4, No 1, Apr-Jun 2010, Pages: 41-43

tential are more mature when assessed for markers such as protamine content, ability to resist DNA fragmentation, apoptotic marker such as TUNEL or by acridine orange (13). In addition we showed that sperm selected with the zeta method results in higher fertilization rate and possibly higher chance of pregnancy (13). In this study, we report full term pregnancy which resulted in birth of a healthy baby, after eleven unsuccessful ICSI/IVF cycles using DGC/Zeta selected sperm.

## Case Reports

A 38 years old woman and her 39 years old husband referred to Isfahan Fertility and Infertility Center for ICSI/IVF cycle. Following, an ectopic pregnancy in 1998, this couple had undergone eleven previous unsuccessful ICSI/IVF despite successful embryo transfer. Patient history also showed two previous abortion including one twin baby. Following a review of patient history and counseling, the couple was advised to undergo the ICSI procedure using the novel DGC/Zeta sperm selection procedure.

Following semen collection, the sperm volume, density and motility were 3.5 ml, 70 million per ml and 55% respectively by WHO guidelines (14). The sperm was initially processed by density gradient centrifugation (DGC) procedure followed by the Zeta method according to our pervious study (13). Also TUNEL assay, Chromomycin A3 (CMA3) and papanicolaou staining applies for assessment of DNA fragmentation, protamine deficiency and normal morphology (15). Figure 1 shows the result of these tests on semen sample, after DGC and DGC/Zeta procedure. ICSI procedure carried out according to our pervious study (13).



**Fig 1:** Result of protamine deficiency assessed with CMA3 staining (black bars), DNA fragmentation assessed with TUNEL assay (hatched bars) and normal morphology assessed according to strict criteria (white bars) on neat semen, after density gradient Centrifugation (DGC) and DGC/Zeta procedure.

After ovum pick up procedure, twelve oocytes were obtained. Ten oocytes were suitable for insemination with DGC/Zeta selected sperm. 16-18 hour post inseminations, nine out of ten oocytes were fertilized. On day three, eight good quality embryos were obtained. Considering the previous unsuccessful ICSI/IVF attempts, three of eight cell embryos were selected for transfer which resulted in 38 week singleton pregnancy and birth of a healthy girl with 3 kg weight through cesarean procedure. The remaining embryos were vitrified.

## Discussion

This is the first report implementing the DGC/Zeta sperm selection procedure for individuals with repeated ICSI/IVF failure. If we consider selection of appropriate sperm to be accountable for resulted pregnancy, sperm selected by natural barrier during IVF procedure should have also resulted in pregnancy. This suggestion could be explained by the fact that, especially in IVF, fertilization barrier may exclude inappropriate sperm to participate in fertilization. Indeed it has been reported that sperm DNA damage does not preclude sperm from participating in fertilization and possibly early embryo development (16). Therefore, it is likely that in pervious IVF cycles, the sperm in this individual may have lead to formation of embryos from sperm with fragmented DNA possibility resulting in unsuccessful outcome. Like our previous report, in this case, assessment of processed sample after DGC/Zeta reveal that reduced percentage of sperm with DNA fragmentation and increased sperm chromatin integrity and sperm normal morphology which might have resulted in selection of appropriate sperm for insemination of oocytes for this successful pregnancy. Therefore, the result of this case report opens the path for further evaluation of DGC/Zeta sperm selection procedure for couples with repeated ICSI/IVF failure.

## References

- Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*. 1992; 340: 17-18.
- Celik-Ozenci C, Jakab A, Kovacs T, Catalanotti J, Demir R, Bray-Ward P, et al. Sperm selection based on shape properties alone does not preclude the presence of numerical chromosomal aberrations. *Hum Reprod*. 2004; 19: 2052-2059.
- Tavalae M, Razavi S, Nasr-Esfahani MH. Influence of sperm chromatin anomalies on assisted reproductive technology outcome. *Fertil Steril*. 2009; 91: 1119-1126.
- Lewis SE, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. *Cell Tissue*

Res. 2005; 322: 33-41.

5. Avendaño C, Franchi A, Taylor S, Morshedi M, Bocca S, Oehninger S. Fragmentation of DNA in morphologically normal human spermatozoa. *Fertil Steril*. 2009; 91: 1077-1084.

6. Said T, Agarwal A, Grunewald S, Rasch M, Baumann T, Kriegel C, et al. Selection of nonapoptotic spermatozoa as a new tool for enhancing assisted reproduction outcomes: an in vitro model. *Biol Reprod*. 2006; 74: 530-537.

7. Razavi S, Nasr-Esfahani MH, Deemeh MR, Shayesteh M, Tavalae M. Evaluation of zeta and HA-binding methods for selection of spermatozoa with normal morphology, protamine content and DNA integrity. *Andrologia*. 2010; 42: 13-19.

8. Nasr-Esfahani MH, Razavi S, Vahdati AA, Fathi F, Tavalae M. Evaluation of sperm selection procedure based on hyaluronic acid binding ability on ICSI outcome. *J Assist Reprod Genet*. 2008; 25: 197-203.

9. Ainsworth C, Nixon B, Aitken RJ. Development of a novel electrophoretic system for the isolation of human spermatozoa. *Hum Reprod*. 2005; 20:2261-70.

10. Chan PJ, Jacobson JD, Corselli JU, Patton WC. A simple zeta method for sperm selection based on membrane charge. *Fertil Steril*. 2006; 85: 481-486.

11. Kirchhoff C, Schröter S. New insights into the origin,

structure and role of CD52: a major component of the mammalian sperm glycocalyx. *Cells Tissues Organs*. 2001; 168: 93-104.

12. Giuliani V, Pandolfi C, Santucci R, Pelliccione F, Macerola B, Focarelli R, et al. Expression of gp20, a human sperm antigen of epididymal origin, is reduced in spermatozoa from subfertile men. *Mol Reprod Dev*. 2004; 69: 235-240.

13. Kheirollahi-Kouhestani M, Razavi S, Tavalae M, Deemeh MR, Mardani M, Moshtaghian J, et al. Selection of sperm based on combined density gradient and Zeta method may improve ICSI outcome. *Hum Reprod*. 2009; 24: 2409-2416.

14. World Health Organization. Laboratory manual for the examination of human semen and semen-cervical mucus interaction. Cambridge, UK: Cambridge University Press; 1999.

15. Nasr Esfahani MH, Razavi SH, Mardani M, Khajavi NA, Deemeh MR, Tavalae M. The Comparison of Efficiency of Density Gradient Centrifugation and Zeta Methods in Separation of Mature Sperm with Normal Chromatin Structure. *Yakhteh*. 2009; 11: 168-175.

16. Henkel R, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, Menkveld R, et al. Influence of deoxyribonucleic acid damage on fertilization and pregnancy. *Fertil Steril*. 2004; 81: 965-972.

---