Evaluation of Protamine Deficiency and DNA Fragmentation in Two Globozoospermia Patients Undergoing ICSI

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Abstract

The aim of this report has been evaluating protamine content and DNA integrity of two patients with globozoospermia undergoing Intra Cytoplasmic Sperm Injection (ICSI).

Semen analysis was carried out according to WHO criteria. Protamine deficiency and DNA fragmentation was assessed using Chromomycin A3 and sperm chromatin dispersion assay respectively**.** ICSI and chemical activation were carried out on inseminated oocyte.

Both cases demonstrated high degrees of protamine deficiency, while one of the cases indicated high level of DNA fragmentation, too. High fertilization rates were achieved in both cases. However, embryo transfer did not lead to implantation or pregnancy.

Artificial oocyte activation overcomes low fertilization rate reported in cases with high level of protamine deficiency. In the present study, failed implantation in one of the cases may be caused by high DNA fragmentation**.**

Keywords**:** Globozoospermia, ICSI, Protamine Deficiency, DNA Fragmentation

Introduction

Patients with round-headed sperm in their semen, called "globozoospermia", are considered sterile (1). Cause of failed fertilization in these cases is inability of sperm to bind to the zona pellucida and penetrate the oocyte as in normal fertilization or in conventional In Vitro Fertilization (IVF) (2). Patient with 100% round-headed sperm in semen are called "Total Globozoospermia" and patient who contain both normal and round-headed sperm are called "Partial Globozoospermia" (3). (ICSI) is the treatment option for these patients, even though in most cases sperm are unable to support oocyte activation after ICSI, due to absence of sperm associated oocyte activation factor (SAOAF) (4). The aim of this report has been evaluating protamine content and DNA integrity of two patients with globozoospermia undergoing ICSI.

Case Report

Semen samples were obtained from two patients undergoing ICSI at Isfahan Fertility and Infertility center. Following semen collection, both of the samples were assessed by routine semen analysis via light microscopy according to WHO guidlines. For morphological analysis, semen smears were stained with papanicolaou (5). Slides also were prepared for assessment of DNA fragmentation using Sperm Chromatin Dispersion (SCD) test according to Fernandez procedure (6), protamine deficiency by Chromomycin A3 staining

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and, also Sperm preparation, ICSI, oocyte artificial activation and embryo culture was carried out according to Nasr-Esfahani, 2007 (7).

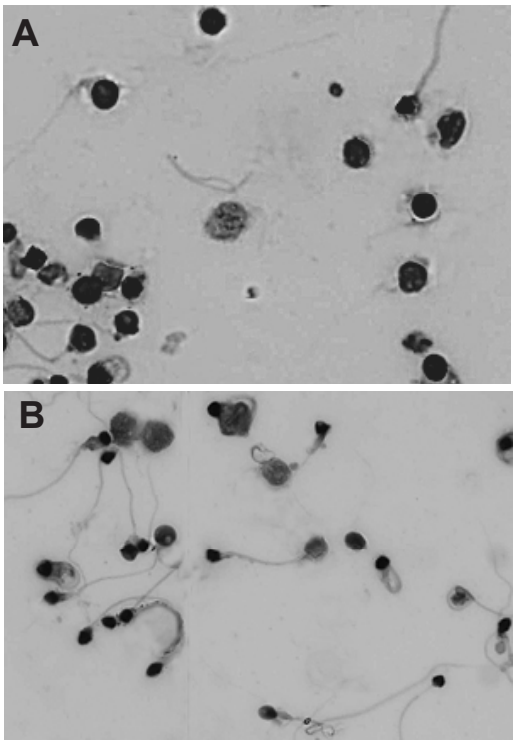

Fig 1: Light microscopy image of round-headed sperm in total (A) and partial globozoospermia (B).

Results

Morphological analysis with Papanicolaou staining indicated that in the first case 98% of semen sperm had morphological abnormalities including 85%, 20%, 18% round-headed sperm, neck and tail anomalies, respectively. But in the second case 100% of sperm had morphological abnormalities including 100%, 40%, 30% round-headed sperm, neck and tail anomalies, respectively (Fig1). Fluorescent staining with CMA3 indicated 87% and 95% CMA3 positivity in the first and second cases, respectively.

Result of SCD test for assessment of DNA fragmentation indicated 28% and 65% of DNA fragmentation, respectively. The results of ICSI procedure in both cases are presented in Table 1.

Discussion

In this report, morphological analyses of both cases reveal severe head abnormality in both cases. In the first case 85% of sperm were round-headed while in the second case 100% were round headed (Fig 1).

Therefore the first case was labeled as "Partial globozoospermia" while the second case was labeled as "total globozoospermia".

Sperms with these levels of abnormality may lead to fertilization failure. This mainly attributed to absence of SAOAF associated with acrosome or perinuclear theca (8). In this study fertilization rate in the first and second case were 60, 76%, respectively. This high fertilization rate was mainly due to chemical activation carried out in this study. In both cases 3 embryos were transferred that did not lead to implantation or pregnancy (Table 1). Except in cases, total fertilization failure usually takes place. It is believed that the success of ICSI depends on chromatin and DNA integrity rather than sperm morphology (9). Sperm protamine deficiency and DNA integrity have been shown to have significant influence on ART outcomes (10). Previous studies implied that in globozoospermia chromatin compaction

Table 1: Result of ICSI procedure in two globozoospermia patient

	No. Oocyte	No. MII oocyte injected	No. 2PN	No. 2-cell (day 2)	No. 8-16 (day 3)	pregnancey
Case 1 (partial Globzoospermia)	11	10	6	4	3	No
Case 1 (partial Globzoospermia)	17	16	13	10	10	No

appear to be disturbed (3). In current study chromatin maturity was assessed by CMA 3 staining which reveals presence or absence of protamine, the main protein responsible for chromatin condensation.

The results of CMA3 staining revealed that in the cases with partial and total globozoospermia 87% and 95% of sperm have protamine deficiencies.

The results of SCD assay also revealed that in the cases with partial and total globozoospermia 28% and 65% of sperm show some degree of DNA fragmentation, respectively.

Although there is no cut off point reported for SCD assay, but the level of DNA fragmentation is different in our two patients.

This difference is unlikely to be due to protamine deficiency, since both cases revealed similar levels of protamine deficiency. Other factors such as external stress as seen in reactive oxygen species or heat may account for this difference.

The results of this report like pervious studies reveal that globozoospermia occurs with high level of protamine deficiency (11). However, in this study fertilization failure may have been overcome by chemical activation. While, high level of DNA fragmentation may account for the failed implantation in the second case.

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