Relationship between ICSI Outcome and DNA Fragmentation Using a Halosperm Kit

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INTRODUCTION

Assisted reproductive technology (ART) is composed of methods that aim for pregnancy and birth. Intrac- aterus insemination (IUI), in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the most common techniques in ART [1]. There are many aspects for the success of ART, without doubt the quality of oocytes and sperm are the most important factors [2, 3] Semen analysis is a routine test to evaluate sperm quality. Despite some pitfalls, the test result is generally acceptable and considered reliable in the assessment of male fecundity [4]. Nevertheless, semen analysis is subjective and sperm parameters vary based on patient conditions. However, they do not always reveal the quality and health of sperm [5]. Sperm DNA integrity is essential for accurate transmission of genetic information [3]. Apoptosis, reactive oxygen species (ROS) and abnormalities in chromatin packaging could be major sources for disintegrated sperm DNA [6, 8]. It seems that genetic abnormalities in the paternal genome are one of the main causes for early pregnancy loss (EPL) [9]. Evidence suggests that a negative correlation with fertility problems and downtime in the organization of genetic material in its sperm nucleus both in terms of In vitro and in vivo [10, 11]. To overcome this problem different methods have been proposed that might be more reliable and valuable than routine semen analysis. These methods evaluate sperm chromatin and DNA integrity related to male fecundity. The sperm chromatin structure assay (SCSA), terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling assay (TUNEL), the single cell electrophoresis assay (COMET), and the sperm chromatin dispersion (SCD) test may reveal more defects in sperm competency [12-15]. These tests are to be correlated with sperm parameters in routine semen analysis [10]. The DNA fragmentation index (DFI) can be used as an independent predictor of fertility in couples undergoing ART [16]. Recently, an easy and fast diagnostic test based on sperm chromatin dispersion (SCD) [15], the Halosperm Kit has been developed. Sperm with fragmented DNA fail to produce the characteristic halo in SCD. Normal sperm without DNA fragmentation nucleoids with large halos of spreading DNA loops [17]. It is postulated that if the sperm DNA fragmentation (DF) value exceeds 30%, sperm quality decreases significantly [18]. Based on a DF quantitative value, it may be possible to choose the appropriate technique in infertility clinics. On the
other hand, using the DF value for all patients is not cost-effective; therefore choosing patients based on their sperm parameters for the DF test is advisable. We investigated the prevalence of sperm DNA damage in a group of infertile men with both normal and abnormal semen parameters in order to elucidate whether Halosperm analysis can add to the information obtained by routine semen analysis in explaining the causes of infertility. In this regard, we have analyzed the relationship of ICSI outcome with sperm DF by using the Halosperm Kit in patients who were candidates for ICSI.

MATERIALS AND METHODS

This was a cross-sectional study performed on 89 infertile men whose wives were completely healthy. The Center’s Ethical Committee approved the study. Patients underwent their first ICSI attempt because of male factor infertility or unsuccessful IVF and IUI procedures, and who referred to Mehregan Clinic and Fatemeh Zahra Fertility Center in Babol, Iran. After preparing semen samples by the swim up method, they were evaluated using a Halosperm Kit to determine the level of DF. Samples for ICSI were classified in two groups: i) patients whose sperm fragmentation level was >30% were considered to have high fragmentation (HFG) and ii) patients whose sperm fragmentation levels was ≤30% were considered to have low fragmentation (LFG) [17]. Participating patients gave consent to participate in the study.

Sperm analysis: Both the semen analysis according to World Health Organization guidelines (WHO, 1999) and DF assessment were performed on the day of oocyte pick-up. Specimens were collected with assistance of the female partner after 3-5 days of sexual abstinence and analysis were performed after liquefaction, by using a light microscope (Olympus/Japan). Sperm concentrations, morphology and motility were assessed before and after semen preparation. Sperm morphology was assessed according to Kruger’s strict criteria after Papanicolaou staining. Sperm concentration was assessed by a Makler counting chamber (Bruckberg, Germany). Sperm motility was classified as either grades A, B or C and at least 100 spermatozoa were scored with a 40x objective. Total motility was calculated as the total of A and B motility rates.

Swim up: Semen samples were diluted at a 1:3 ratio with Ham’s F10 medium without albumin (Sigma Aldrich, USA) and centrifuged for 10 min at 1500 rpm. After centrifugation, the supernatant was discarded. Next, 1 ml of Ham’s F10 medium that contained 20% human albumin (Marburg, Germany) was layered on the pellet. The spermatozoa were allowed to migrate for 20 min at 37˚C and 5% CO2. After 20 min, 0.5 ml of the supernatant were gently aspirated and placed in 5 ml conical tubes.

Assessment of sperm DNA fragmentation (DF): Sperm DF was assessed after semen preparation using the Halosperm Kit (Parque Tecnológico de Madrid Spain). The semen samples were diluted to a concentration of 20 million sperm per ml. Then, spermatozoa were immersed in agarose microgel and spread on the slide. Samples were denatured with an acid and lysis solution, dehydrated and stained with Diffquick. Sperm with large halos (thicknesses that were similar or larger than the length of the smallest diameter of the core) and sperm with mediumsized halos (thickness greater than 1/3 of the smallest diameter of the core and less than the smallest diameter of the core) were classified as 'spermatozoa having no fragmentation' (Figure 1). Spermatozoa with a small halo (thickness similar or smaller than 1/3 of the smallest diameter of the core) and those with no halo were classified as spermatozoa with DF (Figure 2).

Fig. 1: Image of patient's semen in LFG. (a) Sperm with large halo; (b) Sperm with medium-sized halo.
Fig. 2: Image of patient's semen in HFG. (a) Sperm with no halo; (b) Sperm with small halo.

Ovarian stimulation and ICSI procedure:
The standard long protocol of the pituitary suppression with gonadotropin releasing hormone (GnRH) was used in all patients. After 17 days of oral contraceptive, pre-treatment buserelin subcutaneous injection (Superfact; Hoechst, Frankfurt) was administrated at a daily dose of 0.5 cc until hMG (Pergonal; Serono, S.p.A., Rome) initiation on the second or third day of next cycle. On the day of initiation of hMG, the dose of buserelin was modified to 0.25 cc until hCG injection. The hMG was administrated at a daily dose of 150-300 IU for 6-7 days. The dose was modified according to the response. Transvaginal ultrasound was performed every 2-3 days to evaluate follicular size, number and quality. When the largest measured follicle(s) reached a maximum mean diameter of 18-19 mm, 10000 IU hCG (Pregnyle, Organon, Darupakhsh) was administrated intramuscularly. Oocytes were retrieved 36-38 hours after hCG injection through a transvaginal route. Immediately after the collection, oocyte cumulus complexes were put in ham's F10 medium containing 0.1 mg/ml hyaluronidase (Sigma, Aldrich Co., Germany) for maximum of 30 seconds. With handdrawn glass pipette, oocytes were demuded completely. ICSI was performed in all MIIoocytes with normal appearance. Maximum of four injected oocytes were transferred to a drop of 20-30μl conventional GII medium (Vitrolife, Sweden), and it was incubated at least 6 hours in 6% CO2 air under mineral oil (Nidoil, Nidacone, Sweden).

Injected oocytes were evaluated under an inverted light microscope (TE 300, Nikon, Japan) at × 200 magnifications for presence of two pronuclei (PN) 16-20 hours later. Zygotes with 1, 3 PN or more and 2PN with significant difference in size and vacuolated zygotes were eliminated. Zygotes were evaluated as good; if two pronuclei were centralized, apposed, and polarized. Zygotes let further culture for 24-30 hours. Embryos were assessed based on number of blastomeres and percentage of cytoplasmic fragments. If there were four-cell embryos with <5% fragmentation, maximum of three embryos were transferred into the uterine cavity. Size of blastomeres and status of nucleus in blastomeres were not considered. Assisted hatching was performed mechanically in all pre-embryos, except for the cases that zona pellucida was hatched easily. Embryos were transferred in maximum of 30-40 μl media (GII) by using ultrasound guided catheter (Frydman, CCD, France) and expelled at midpoint of uterine cavity. The quality of embryo transfer (bloody cervix only) was recorded in each case.

Luteal supplementation Outcome measures:
The luteal phase was supplemented with a vaginal administration of 800 mg of natural progesterone (Cyclogest; Hoechst) daily beginning 24 hours after ovum pick-up, and continued until 8 weeks’ gestation if pregnancy was occurred. Pregnancy test (β-hCG) was performed 15-16 days after hCG administration. A positive test of pregnancy was followed with an ultrasound to detect gestational sac at 5 weeks after menstrual age.

Statistical analysis:
Statistical analysis was performed using SPSS software (version 18, SPSS Inc., Chicago, IL, USA). Categorical data were analyzed by the chi-square test and continuous data by the independent sample t-test. A p-value less than 0.05% was considered statistically significant.

RESULTS AND DISCUSSIONS
Characteristics of patients participating in the study can be seen in Table 1. In the connection with the characteristics of sperm, sperm concentration, sperm motility and sperm cells in the patients studied, according to the results reported in this paper are applicable to this study, average parameters showed a statistically significant difference between the two groups in the LFG and HFG. P-Value in the each of the parameters of concentration, sperm motility and normal forms are as follows (0.008, <0.001 and <0.001).
There is no significant difference between the mean number of Oocytes collected and fertilized injection in the both LFG and HFG. Fertilization of Oocytes was observed even in the samples with high DNA fragmentation. ICSI outcome was compared between LFG and HFG (Table 2). With regard to the first embryo transfer in patients fertility results were considered in the patients. The low percentage of DNA fragmentation in the group of 53 patients with LFG, the result was positive In the 12 patients and was pregnant. The high percentage of DNA fragmentation in the group of 36 patients with HFG, the positive result was observed In the 6 patients, and pregnancy. There are no statistically significant differences in the parameters. Finally, fertility and pregnancy showed no differences between LFG and HFG (22.6%, 16.7%).

Table 1: Demographic characteristics of patients.

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<th>Age 0f women (years) (mean)</th>
<th>Age 0f men (years) (mean)</th>
<th>Infertility length (years) (mean)</th>
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Discussion:

50% of infertility problems are due to male infertility factors. Most fertility clinics evaluate semen samples by conventional analysis, which does not ensure the absence of male infertility factors [19]. DF is necessary to assess the level of that in men with normal semen parameters, seen a high level of fragmentation [20]. The data indicate that the genome father Abnormality irreversible impacts on blastocyst development even when sperm is injected directly into the oocyte by ICSI [21, 22]. Furthermore, when men are a high percentage of immature sperm, the pregnancy rate was significantly reduced dramatically. In total, average, and high DFI have a predictive value of 100% on beginning pregnancy or defect in it [5]. Factors influencing have shown on embryo development to the Blastocyst stage as a cause of infertility with some negative effects. It's important to understand the factors on blastocyst development in that only about 20% of human embryos have been replaced after the transfer to the uterus. The pregnancy rate declined significantly when men have a high percentage of immature sperm. In addition, it seems that in terms of In vivo embryo Ebner taxes directly associated with male factor the results of the evaluation show waste transfer into the uterus after birth or abortion without involving the female factors. Therefore, its role should be reviewed to assess sperm quality blades at low blastocyst development and pregnancy failure in the beginning, these factors are associated with a significant number of blastocysts [23]. Currently, there is no an uncertainty about the impact of ART on the results of the damage DNA. Some are studies suggesting a negative effect of DNA damage on fertilization rate, the quality and pregnancy rates and others not associated with the outcome of IVF and ICSI. Gandini A study in 2004 showed that there is no relationship between SCASA parameters in a pregnancy and those who did not; pregnancy was possible, even with a high level of DFI [3]. Karydis and Yilmaz did not report any correlation between pregnancy and the DF method ICSI (24). In a 2007 study by Mona Bungum, the groups were treated with ART, including IUI and IVF and ICSI in terms of the parameters of the SCASA. The results showed that the clinical pregnancy decreased significantly in the group treated with IUI in cases where more than 30% of DFI compared with patients who are less than 30% DFI. Also, in general, the results of ICSI were significantly better than IVF the group has more than 30% DFI.

The study showed that infertile men should be checked by a sperm analysis test as a supplement when DFI greater than 30%, ICSI should be considered as a method of choice [16]. In 2008, Mona Bungum and colleagues determined the SCASA parameters fresh sperm samples were prepared by density gradient. The results showed that there is no significant difference in sperm DFI prepared for these groups in the groups treated with IUI IVF and ICSI clinical pregnancy was created in couples and those who create and those who could not be created. Therefore, SCASA parameters in the prepared sperm can not predict pregnancy outcome in ART gradient method. While the raw samples of these factors can be predict pregnancy outcome [25]. Micheal Virro in a study in 2004 showed that the two groups treated with IVF and ICSI the men had a DFI more than 30%, blastocyst formation rate is lower and decreases the chance of pregnancy the group had a 30% lower than the DFI them. When sperm parameters were analyzed in terms of the standard, 18% of men and 28% of them were still DFI in the high risk group for blastocyst development and pregnancy failure in beginning and spontaneous abortions seen in spouses of patients [18]. In 2000, Marcello Spano The study showed sperm chromatin packaging for male fertility is a necessary condition he reported a positive relationship between the DFI and reduced fertility and early
embryonic division (10). Larson and Henkel (2004) showed a negative association between pregnancy rate and the balance [26, 27]. Our study showed no relationship between pregnancy rate and the percentage of DNA fragmentation. These results suggest that embryo development is unaffected by the third day, as the evolution of the embryonic genome is activated by DNA fragmentation after the third day [28] as the fetus is able to repair damaged DNA [29]. Also, these results may be due to the fact that during the process of ICSI, the sperm are selected with better features are so much lower DF as a result, the embryos are transferred [17] in total, recommended further studies to determine the relationship between sperm DNA fragmentation with pregnancy outcomes and efficiency of ICSI.

REFERENCES