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The quality of sperm preparation medium affects the motility, viability, and DNA integrity of human spermatozoa

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Abstract

AIM:

The goal was to compare the effects of three different sperm preparation media on sperm motility, viability, and DNA integrity of semen samples from normozoospermic men.

METHODS:

A total of 15 normozoospermic males were included in the study. The semen analysis (SA) was performed in accordance with the WHO guidelines (2010). After SA, each sample was divided into three aliquots, and swim-up was performed with three different sperm preparation media (Sperm Preparation Media, Origio, Denmark; Ham's F10, Biochrome, Berlin, Germany; and VitaSperm™, Innovative Biotech, Iran). Sperm motility, viability, and DNA fragmentation were evaluated at 0, 1, 2, and 24 h after swim-up.

RESULTS:

There were no significant differences, at any time intervals, in the total sperm motility between the different sperm preparation media. However, the rate of progressive motility was significantly higher in spermatozoa prepared using the media from Origio in comparison with VitaSperm™ ($P = 0.03$), whereas no significant difference was found against Ham's F10 medium. No significant differences in sperm viability were seen between the media products. However, 1 h after swim-up, the extent of sperm DNA fragmentation was lower in the medium from Origio versus VitaSperm™ ($P = 0.02$).

CONCLUSIONS:

The data showed that the quality of medium for preparation of semen samples from normozoospermic men significantly affects the performance of spermatozoa in assisted conception programs.

KEY WORDS: DNA fragmentation, sperm motility, sperm preparation media, sperm viability

INTRODUCTION

The need for assisted reproduction technology (ART) procedures for the establishment of pregnancies has steadily increased worldwide. Around 7% of all annual births are thought to be established by ART, which corresponds to 1 million treatments.[1] Therefore, it is of vital importance that an efficient sperm

preparation technique used for retrieval of high-quality spermatozoa contributes to the creations of high-quality embryos, with high implantation potential.[2] The main goal with the sperm preparation is to yield highly motile spermatozoa with good morphology and low DNA fragmentation rates, criteria that support the subsequent development of high-quality embryos. The sperm DNA integrity affects fertilization,[3] embryo development, pregnancy outcome,[4,5] miscarriage rates,[2] and abnormalities in the offspring[5] after both *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI). In animals, development of cancer and shorter lifespan in their offspring are related to the injection of spermatozoa with high DNA fragmentation rates.[6,7]

The recovery of a good proportion of high-quality spermatozoa depends on the quality of the semen sample, the sperm preparation method, time and storage temperature of the prepared sperm suspension.[8,9,10] The choice of sperm preparation medium also interferes with the recovery of high-quality spermatozoa, which affects the treatment outcome.[11] An effective sperm preparation method yields a high number of motile spermatozoa with good morphology and quality, which may convert more complex treatment, such as ICSI, into more simple techniques of IVF or intrauterine insemination (IUI) for a more natural and cost-effective treatments. It is also important to evaluate the integrity of sperm DNA after the sperm preparation technique since morphologically normal spermatozoa still may have DNA damage.[12,13] Currently, there are several techniques for detection of sperm DNA fragmentation. The sperm chromatin dispersion (SCD) assay evaluates DNA fragmentation and has the same predictive values such as the sperm chromatin structure assay (SCSA) and TdT-mediated dUTP-biotin nick end labeling (TUNEL).[14] In addition, this assay is easy, rapid, accurate, and not requiring sophisticated instruments. The aim of this study was to compare the effects of three different sperm preparation media on viability, motility, and DNA integrity of spermatozoa after preparation of normozoospermic samples.

METHODS

Patients

This case study involved 15 normozoospermic specimens from men undergoing infertility. This study was done from July 2014 to February 2015. All the patients signed the consent form. This study was approved by the Institutional Review Board.

Semen analysis

The semen samples, according to the WHO guidelines,[15] met the inclusion criteria. Khalili *et al*. chamber was used for determination of the sperm count and motility characteristics.[16] The following criteria were applied for evaluation of sperm motility: Rapidly progressive spermatozoa were considered as Grade a. Grade b spermatozoa were slowly progressive, Grades c and d were dedicated to no progressive motility and immotile spermatozoa, respectively. Morphology was evaluated using Papanicolaou method.[9] Three culture media were selected: (a) Sperm Preparation Media (Origio, Måløv, Denmark), (b) Ham's F10 (Biochrome, Berlin, Germany) supplemented with 5 mg/ml human serum albumin, and (c) Sperm Washing Medium (VitaSperm™ Innovative Biotech, Tehran, Iran), where 5 mg/ml HSA was added. Sperm motility, viability, and DNA fragmentation index (DFI) were evaluated at 0, 1, 2, and 24 h after preparation. Sperm viability and DFI were assessed after staining with eosin-nigrosin, and the SCD technique, respectively.

Sperm preparation

The samples were prepared by direct swim-up method. Briefly, 1.2 ml of each culture media was placed into a sterile conical tube, after which 1 ml of the semen was slowly placed under the medium at the bottom of the tube. The swim-up was performed at 37°C in an incubator at 45° angle for 45 min. Thereafter, 1 ml of the upper layer was removed, and 2 ml of the same sperm preparation media was added to the test tube. After centrifugation at 400 g for 7 min, the pellet was resuspended in 0.5 ml preincubated media, the tubes closed tightly, and the sperm suspension analyzed at the aforementioned time intervals.

Sperm chromatin dispersion test

The SCD test was performed according to Fernández *et al*.[17] Glass slides were coated with 0.65% (w/v)

agarose and 70 μl of a low-melting 1% (w/v) agarose was mixed with 30 μl of the sperm suspension. Thereafter, 50 μl of this mixture was placed on the precoated glass slides and kept at 4°C for 4 min. Next, the slides were placed in a denaturation solution (0.08 M HCL) for 7 min at room temperature (RT) in the dark. These slides were placed in 0.4M Tris, 2-mercaptoethanol containing 1% (w/v) sodium dodecyl sulfate (SDS) and 50 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.5), for 10 min at RT. After incubation, the slides were placed in a lysing solution (0.4M Tris, 2M NaCl, and 1% [w/v] SDS, pH 7.5) for 15 min and were finally washed in a Tris-borate-EDTA buffer (0.09 M Tris-borate and 0.002 M EDTA, pH 7.5) for 2 min at RT. The slides were dehydrated in ascending ethanols (70%, 90%, and 100% [v/v]) for 2 min and left to dry at RT.

For staining the slides, Wright's stain was mixed with a phosphate buffer solution at a ratio of 1:1. This mixture was layered on the slides placed in running water until the excess stains washed away and left to dry at RT. The DNA fragmentation rate was related to the halo around the sperm head and categorized into four groups: (a) Big halo, when the halo size was more than the minor diameter of core width (without DNA fragmentation), (b) small halo, with size smaller than one-third of the minor diameter of core width, (c) medium halo size was considered between large and small halos, and (d) no halo. Large- and medium-sized halos have no DNA fragmentation while small size halos or no halos have a fragmented DNA [Figure 1].

Statistical analysis

The data are shown as mean ± standard deviation and median (minimum–maximum). The Shapiro test was used for evaluating normal distribution of data. One-way analysis of variance followed by Tukey as a posttest and Kruskal–Wallis test was applied to compare sperm parameters between media at one time point. All hypotheses were considered two-tailed, and $P < 0.05$ was considered statistically significant.

RESULTS

There were no significant differences in progressive motility between the different sperm preparation media after swim-up at 0, 1, and 24 h. However, the rate of progressive motility was significantly higher in spermatozoa prepared by Sperm Preparation Media, when compared to VitaSperm™ (68.3 ± 11.5 vs. 58 ± 9.8, $P = 0.03$) [Figure 2]. There were insignificant differences in motility or viability between the different groups [Tables 1 and 2]. In addition, there was no difference in the percentage of normal morphology between Sperm Preparation Media, VitaSperm™, and Ham's F10 after swim-up (15.9 ± 2.1, 16.4 ± 3.2, and 15.8 ± 2.3, respectively). The sperm DFI increased significantly 1 h after swim-up in VitaSperm™ versus Sperm Preparation Media (3 ± 2.7 vs. 1.1 ± 0.8, $P = 0.02$); however, there was insignificant difference in comparison to Ham's F10 (1.7 ± 1.1). However, the DFI increased significantly over time in the VitaSperm™ medium versus other two media [Figure 3].

DISCUSSION

The quality of the prepared sperm suspension affects the outcome of all types of inseminations, whether it is for IUI, IVF, or ICSI. After sperm preparation, the recovery of motile spermatozoa affects the decision of which method would be the most suitable and efficient for the couple. If there is a high recovery of highly motile spermatozoa with good morphology and low DFI, the prepared sperm suspension is suitable for more physiological methods, such as IUI or IVF. Whereas, borderline or male factor samples might be more suitable for ICSI. The handling of the semen sample, choice of sperm preparation method, and storage temperature of the prepared sperm suspension affect the recovery rates of sperm, their fertilization ability, the integrity of the sperm DNA, their ability to support embryo development and the implantation rate.[3,4,5,9] The effects of different sperm preparation techniques on sperm parameter values and the DNA integrity have been evaluated in several studies.[8,18,19,20] It seems that the yield of morphologically normal spermatozoa with good chromatin stability is obtained after preparation of the semen sample by high-density gradient centrifugation in comparison with conventional swim-up and glass wool filtration methods.[19]

In this study, we evaluated the efficacy of three different sperm preparation media on motility, viability, and

DNA integrity of human spermatozoa at different time intervals after sperm preparation. Our data showed that the nature of the sperm preparation medium affects sperm motility and the DNA integrity of the prepared spermatozoa. Depending on the quality, the storage temperature, and the incubation time of the sperm suspension, as well as the preparation medium, the level of sperm DNA fragmentation varies.[9,10] Oxidative stress and lipid peroxidation have been suggested to affect sperm motility[21,22] by inducing damages to the axoneme and depletion of intracellular adenosine triphosphate, which reduces the sperm motility.[23,24,25] It is thought that the main product of oxidative stress, 8-hydroxy, 2′deoxyguanosine, also causes most of the damage to the sperm DNA.[26] The extent of sperm DNA fragmentation could be used as a sperm quality marker, which predicts the fertilization capability of the prepared spermatozoa. The main causes to sperm DNA fragmentation are thought to be related to failure in the chromatin remodeling during spermiogenesis or an increase in reactive oxygen species (ROS) levels due to oxidative stress.[27] However, the impact of sperm DNA fragmentation on fertilization has still not been confirmed.[5,28] However, a defect in sperm chromatin stability might affect the sperm cells' ability to penetrate the rigid zona pellucida.[3,29]

The SCD test is based on the halo formation of dispersed DNA loops after acid denaturation, where spermatozoa with a high DNA fragmentation rate do not create a halo.[9] It has been shown that the SCD gives similar results to that of both the SCSA and the TUNEL assay and that it can be used as a prognostic tool for sperm DNA fragmentation rate.[14]

Oxidative stress in subnormal specimens is induced by other types of contaminating cells, immature and bad morphology spermatozoa, as well as the sperm wash method.[8,15,30,31,32,33] We, therefore, used normozoospermic samples for this study, to reduce potential sources of oxidative stress to spermatozoa and for accurate interpretation of the results.[9,34] The method of choice for preparation of semen may affect the production of ROS, thereby inducing oxidative stress.[35] However, Younglai *et al*. showed that there was no significant difference in DNA fragmentation rate in spermatozoa prepared by direct swim-up from the semen sample or after prewash of the semen sample.[36] In this study, all samples were prepared by the direct swim-up method; thus, the findings should only be related to the quality of the sperm preparation media. Formation of free radicals and oxidative stress also seem to play a physiological role in sperm capacitation and fusion between the spermatozoa and the oocyte.[30] Antioxidants, such as ascorbic acid and tocopherols, seem to prevent the detrimental effects that ROS have on sperm motility and DNA fragmentation.[34,37] The inclusion of antioxidants in sperm preparation media might, therefore, reduce the detrimental effect that ROS have on sperm during the preparation procedure.

CONCLUSIONS

The quality of the sperm preparation media affects the sperm recovery of high-quality spermatozoa which subsequently may influence the ART outcomes.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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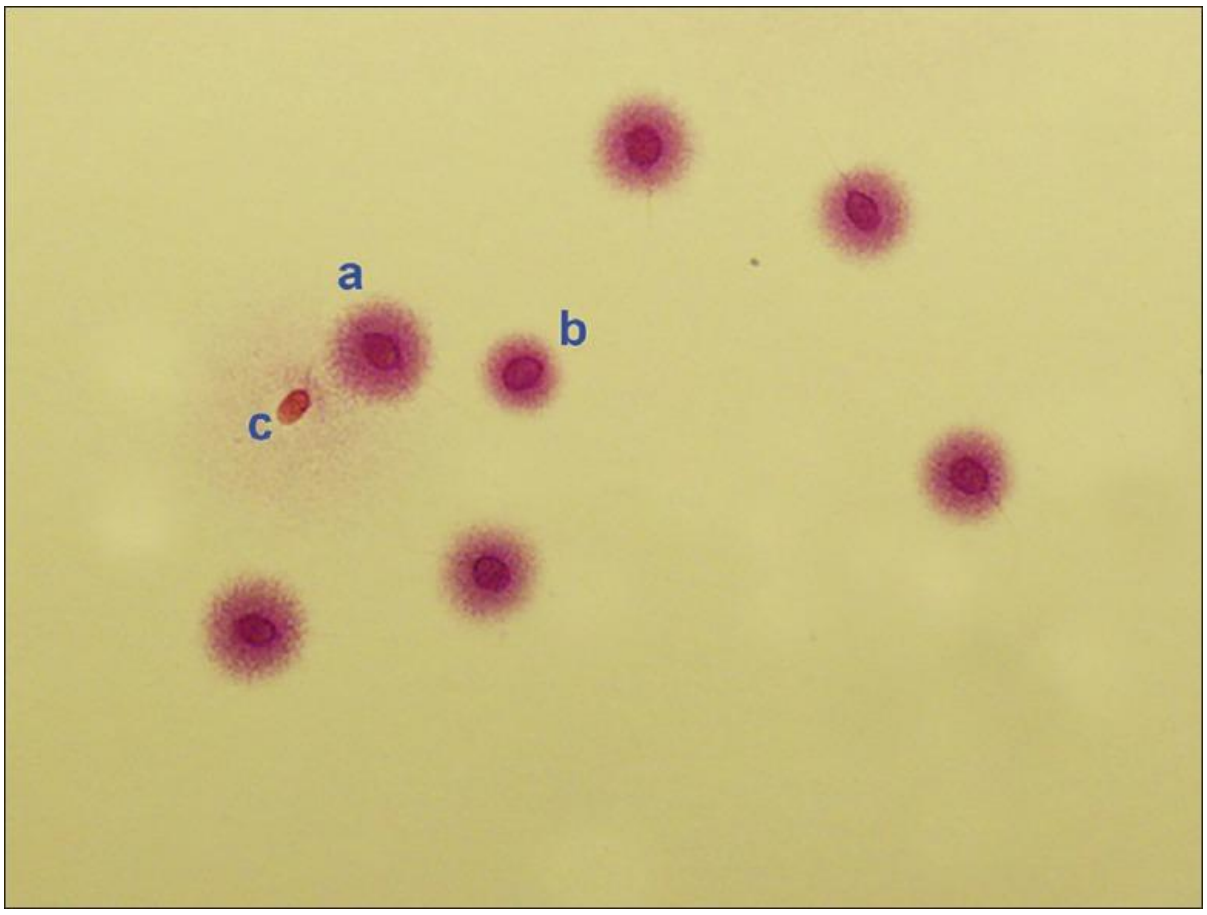
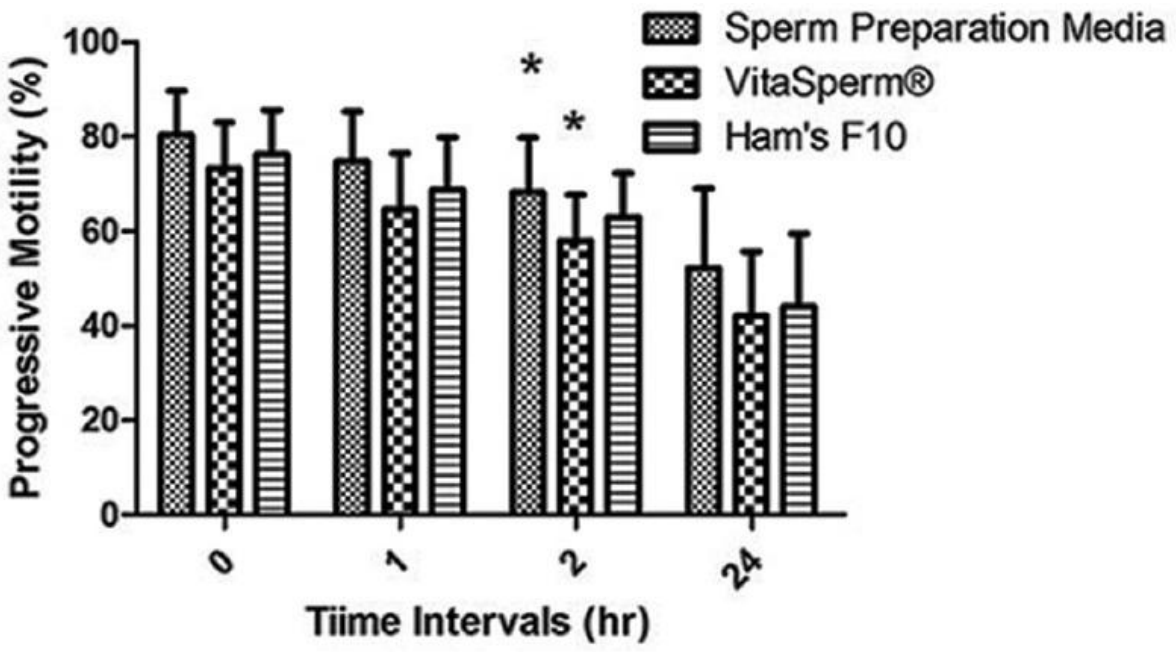
Figures and Tables

Figure 1



Sperm chromatin dispersion test. (a) A big halo represents no DNA fragmentation, (b) a medium halo represents no DNA fragmentation, and (c) no halo indicates spermatozoa with DNA fragmentation

Figure 2



The percentage of progressively motile spermatozoa after swim-up. *P = 0.03

Table 1

Culture media	Time intervals (h)			
	0	1	2	24
Sperm preparation media	87.1±8.7 88 (64-98)	83±10.5 86 (52-93)	77.2±11.9 79 (55-98)	65.1±14.5 69 (32-84)
VitaSperm®	82.1±10.8 85 (53-93)	76±13.5 79 (38-93)	77.2±12.5 76 (38-86)	61.5±12.8 65 (24-76)
Ham's F10	86±9.6 88 (65-96)	80±11.5 80 (58-94)	76.4±10.3 78 (55-95)	60.1±14.2 60 (32-83)
P	0.3	0.2	0.4	0.5

Data are shown as mean±SD, median (minimum-maximum). SD=Standard deviation

Total number of motile sperm (%) at different time intervals after swim-up

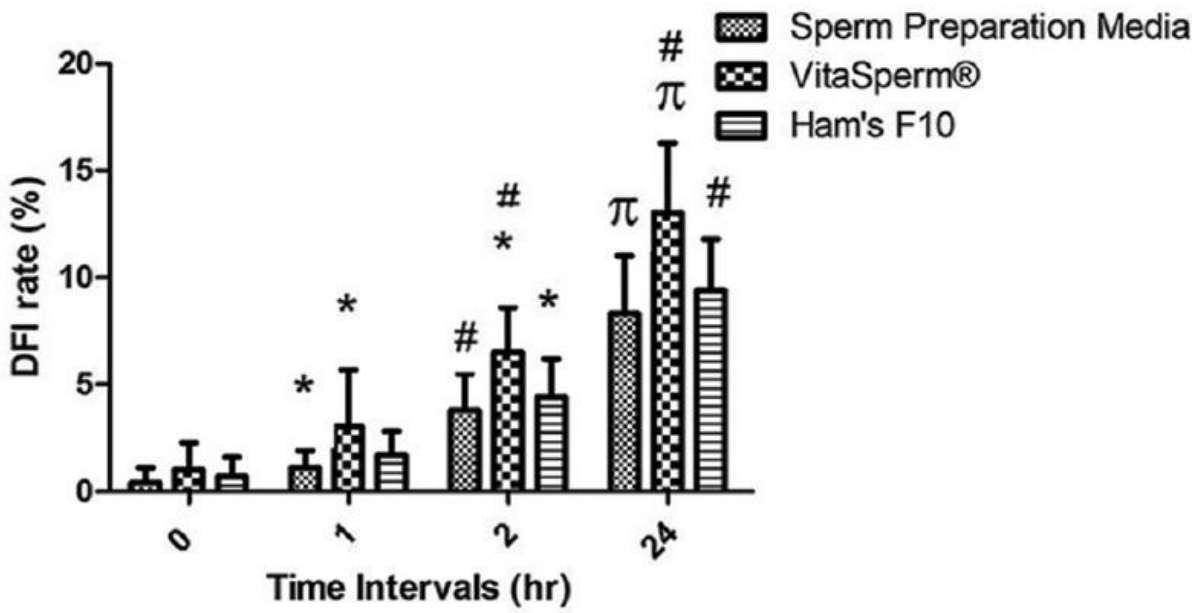
Table 2

Culture media	Time intervals (h)			
	0	1	2	24
Sperm preparation media	98.1±1.6 99 (95-100)	96.2±3.2 98 (88-99)	94.8±4.1 96 (82-98)	93.5±4.8 95 (80-98)
VitaSperm®	97.8±1.5 98 (94-99)	94.8±3.6 97 (87-99)	93.4±4.4 95 (81-97)	92.5±4.5 94 (80-97)
Ham's F10	98±1.7 99 (94-100)	95.8±2.6 97 (90-99)	94.8±2.7 96 (89-98)	91.3±4.5 92 (81-97)
P	0.6	0.3	0.3	0.1

Data are shown as mean±SD, median (minimum maximum). SD=Standard deviation

Sperm viability rates (%) at different time intervals after swim-up using eosin-nigrosin stain

Figure 3



The rate of sperm DNA fragmentation index after swim-up of normozoospermic samples in different sperm preparation media at different time intervals. $^{*}P < 0.05$, $^{\#}P < 0.01$, $\pi P < 0.001$