Middle East Fertility Society Journal 23 (2018) 216–219

Contents lists available at ScienceDirect

Middle East Fertility Society Journal

journal homepage: www.sciencedirect.com

Original Article

A simple, rapid and economic manual method for human sperm DNA extraction in genetic and epigenetic studies

Mahsa Darbandi [a,1], Sara Darbandi [a,1], Hamid Reza Khorram Khorshid [b], Mohammad Mehdi Akhondi [c], Pooneh Mokarram [d], Mohammad Reza Sadeghi [c,*]

[a] Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
[b] Genetics Research Center, The University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
[c] Developmental Biology (Embryology), Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
[d] Department of Biochemistry, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Article history:
Received 23 November 2017
Accepted 15 December 2017
Available online 23 December 2017

Keywords:
Absorbency ratios
DNA concentration
Genetic and epigenetic studies
Protamine
Proteinase K
Sperm DNA extraction
TRIzol

ABSTRACT

Over the recent years, isolation of high quantities of pure, intact, double-stranded, highly concentrated and not contaminated genomic DNA is prerequisite for successful and reliable large scale genetic and epigenetic analysis and also high quantities of pure DNA are required for these types of studies. The DNA extraction methods developed for human somatic cells are not effective for sperm because of the high rating of nuclear compaction and DNA integrity in sperm cells. In the present study, an economic, reliable and simple method of DNA extraction procedures, were examined to ascertain their relative effectiveness for extracting DNA from human sperm. The quality and quantity of the extracted DNA were assessed by spectrophotometric measurements, methylation-specific PCR (MSP) amplification, and gel electrophoresis. This method was applied successfully from sperm samples (N = 50, age = 33.9 ± 7.8 years) with DNAs absorbency ratios of A260/A280 (1.82 ± 0.03) and A260/230 (1.95 ± 0.16). Gel electrophoresis revealed non-degraded and suitable quality genomic DNA for epigenetic and genetic analysis. The purity and quality of this protocol was closer to the optimum value sand no contamination during manual extraction was observed. It concluded that this described method might have a sufficient quality for subsequent molecular analysis and general genetic and epigenetic methods.

1. Introduction

Over the recent years, epigenetic and genetic studies are in progress so isolation of high quantities of pure, intact, double-stranded, highly concentrated and not contaminated genomic DNA is prerequisite for successful and reliable large scale analysis and also high quantities of pure DNA are required. The objective of this work was to evaluate the method for extraction of genomic DNA from sperm samples in terms of DNA quantity, concentration, purity, and integrity as well as utility and applicability for subsequent genetic and epigenetic studies. The organization and compaction of human sperm chromatin are differing from somatic cells [1]. In the somatic cell to decrease the volume of chromatin, nuclear DNA is enveloped around the histones octamer packaged into a solenoid structure. Sperm chromatin configuration is structured by protamines in a particular style to keep the chromatin condensed in the nucleus during transport of the paternal genome in the male and female reproductive tracts [1–3]. Through the spermatogenesis, the most of the sperm cytoplasm loss, the motile tail growth, and somatic-type histones change with protamine to developing disulfide bridges bond [1,3]. Therefore sperm chromatin is denser than (at least six times) the other cells, so the densely packaged genetic information transferred to the embryo [1–3]. Epigenetic has an important part in the regulation of gene expression and altered by the DNA sequence (DNA methylation) or chromatin-associated proteins (i.e., histones) [4]. Distinct the genome, the epigenome is greatly variable between cells reply to cellular and environmental tension [1,5]. These stress can disrupt normal chromatin organization which is vital for fertility and health of the offspring [4]. Thus, for this studies achieving the methods that could extract DNA easily and inexpensively were required. A wide variety of methods have been advanced to extract DNA from several types of human somatic cells [6] but DNA isolation techniques used routinely for human somatic cells have

Peer review under responsibility of Middle East Fertility Society.
* Corresponding author.
E-mail addresses: mahsadarbandi@hotmail.com (M. Darbandi), saradarbandi@hotmail.com (S. Darbandi), hrkk1@uswr.ac.ir (H.R. Khorram Khorshid), mma.akhondi@gmail.com (M.M. Akhondi), Mokaram2@gmail.com (P. Mokarram), sadeghi@avicenna.ac.ir (M.R. Sadeghi).
[1] Equal contribution of Sara Darbandi and Mahsa Darbandi.

https://doi.org/10.1016/j.mefs.2017.12.005
1110-5690/

proven ineffective for sperm [1,7]. The purpose of this article was to provide an economical, simple and reliable method to extract human sperm DNA which could be used in genetic and epigenetic studies.

2. Material and methods

2.1. Study design and sample collection

Semen samples (N = 50, age = 33.9 ± 7.8 years) were collected from men who attended andrology center at Avicenna Infertility Clinic (Tehran, Iran), during April to May 2016. Semen analysis was performed according to the World Health Organization criteria. The patients with azoospermia were excluded from the study. All of the experimental procedures of the study were approved by the Ethics Committee of Avicenna Research Institute and Human Rights Committee. The written consent form was obtained from all of the participants and kept in their medical records.

2.2. Methods

Semen samples were allowed to liquefy for 30 min at 37 °C before analysis with computer-assisted semen analysis (CASA) according to the WHO outline [8]. Samples were analyzed for concentration, motility, and vitality. The total semen was centrifuged at 3400 rpm for 5 min to remove supernatant. The pellets were resuspended and washed twice with 1000 μL sterile phosphate buffered saline (PBS, 0.5 M) at 3400 rpm for 5 min to eliminate cellular and non-cellular components earlier to the addition of lysis buffer. The final pellets were stored at −20 °C.

2.2.1. DNA extraction method

For thawing the samples, the pellet had been allowed to sit at room temperature for 15 min. To make A lysis buffer (10×), 5 ml Tris-HCl 1 M, 1 ml NaCl 5 M and 2.5 ml $MgCl_2$ 1 M should be added to 41.5 ml H_2O. Then 1000 μL of A lysis buffer (1×) was added, vortexed and placed at room temperature for 15 min, to resuspend the cells. This tube was centrifuged at 3400 rpm for 10 min and the supernatant was discarded to remove somatic cells. This step was repeated once again. Then 500 μL TRIzol and 50μL proteinase K were added to the micro tube and strongly mixed by inverting the tube for 40 times. The micro tube was placed in a 56 °C water bath and incubated for overnight. The tube was removed from the water bath and let cool at room temperature. Then 500 μL chloroform was added and mixed strongly by inverting for 40 times until observed white color. Adding chloroform created three separated phase. The tube was located in 4 °C for 15 min and after that was centrifuged at maximum speed for 15 min, 4 °C. The higher clear phase (about 400 μL containing DNA) was removed to a new tube and 800 μL pure cold ethanol and 40 μL cold 3 M sodium citrate were added to simplify DNA sedimentation. It was inverted smoothly until DNA strands form then stored at −20 °C for 1 h. After 1 h the tube was centrifuged at maximum speed for 20 min, 4 °C. The supernatant carefully was empty out. The white DNA pellet was washed twice in 600 μL 70% ethanol then the alcohol solution was removed by reversing the tube to drain. DNA was left at room temperature overnight to more rehydrate in 100 μL Tris–HCl or ddH2O.

2.2.2. Assessment of DNA quantity and purity by spectrophotometer

Quantity and purity of DNA extracted with this method were assessed using a NanoDrops ND-1000 spectrophotometer (PeqLab, Erlangen, Germany) which calculates the concentration of double-stranded DNA (dsDNA). The DNA concentration and ratio of the 260/280 and 260/230 absorbance were automatically measured to define DNA purity. The ratio of absorbance at 260 nm and 280 nm was used to assess protein contamination while the ratio of absorbance at 260 nm and 230 nm was calculated to assess guanidine contamination. The spectrophotometric measurements used for DNA quality assessment with higher values associated with better DNA purity and quantity.

2.2.3. Assessment of whole genomic integrity by gel electrophoresis

The integrity of DNA extracted by this method was assessed by gel electrophoresis. Specifically, 2 μL of each DNA extract was electrophoresed on a 1.5% agarose gel containing nucleic acid gel stain (Smobio, Hsinchu City, Taiwan) and was visualized by U.V. illumination.

2.2.4. Assessment of DNA quality for epigenetic research

We assessed the epigenetic quality by assessing the methylation status of Kelch-like ECH-associated protein 1 (Keap1) in extracted DNAs. Bisulfite-modification of extracted DNAs was performed manually by modifying 2 μg of extracted and denatured DNAs with NaOH and sodium bisulfite to convert unmethylated cytosines to uracil [9,10]. The modified DNA was amplified by MSP using specific primers to distinguish unmethylated (changed) from methylated (unchanged) sequences [11,12]. PCR reactions were performed using the master mix PCR buffer (6μL), primers (50 pmol each per reaction), and bisulfite-modified DNA (50 ng) in a final volume of 12 μL. Reactions were hot-started at 95 °C for 5 min and amplified by Mastercycler Gradient (Eppendorf, Hamburg, Germany) for 35 cycles, followed by a final 4-min extension at 72 °C. Two sets of primers were used separately to check for methylated or unmethylated islands. The human methylated and non-methylated DNA set (Zymo Research, California, United States) were used respectively as methylated and unmethylated DNA controls. Each PCR reaction products were directly electrophoresed on a 1.5% agarose gel, which was later stained with nucleic acid gel stain (Smobio, Hsinchu City, Taiwan) to allow DNA visualization under UV illumination.

2.2.5. Statistical analysis

The mean ± SD of parameters were performed with SPSS software (V. 16, Chicago, Illinois, USA). The Pearson's was used for correlation of parametric variables. $P \leq .05$ was considered to be statistically significant.

3. Results

Total sperm samples were used for DNA extraction. After the extraction procedures, DNA was directly quantified by spectrophotometry. Concentration and total yield of extracted DNA were assessed using the NanoDrops ND-1000 spectrophotometer. No failure was detected in the manually prepared samples and 50 samples were included in this procedure. Descriptive statistics of this method for all samples were summarized in Table 1. As

Table 1
The summarized results indicated the mean ± SD of parameters.

Parameters N = 50	Range	Mean ± SD
Age	20–56	33.9 ± 7.89
Sperm concentration (*10^6)	1–150	51.78 ± 21.95
Volume (mL)	1–7	3.12 ± 1.40
Vitality (%)	18–95	86.90 ± 11.79
PH	7.30–7.60	7.47 ± 0.07
A (%)	0–25	6.90 ± 7.16
B (%)	2–40	29.34 ± 7.40
C (%)	5–30	17 ± 5.24
DNA concentration (ng/μl)	147.20–4157.80	1126.14 ± 810.75
DNA 260/280 absorbance ratio	1.70–1.88	1.82 ± 0.03
DNA 260/230 absorbance ratio	1.70–2.35	1.95 ± 0.16

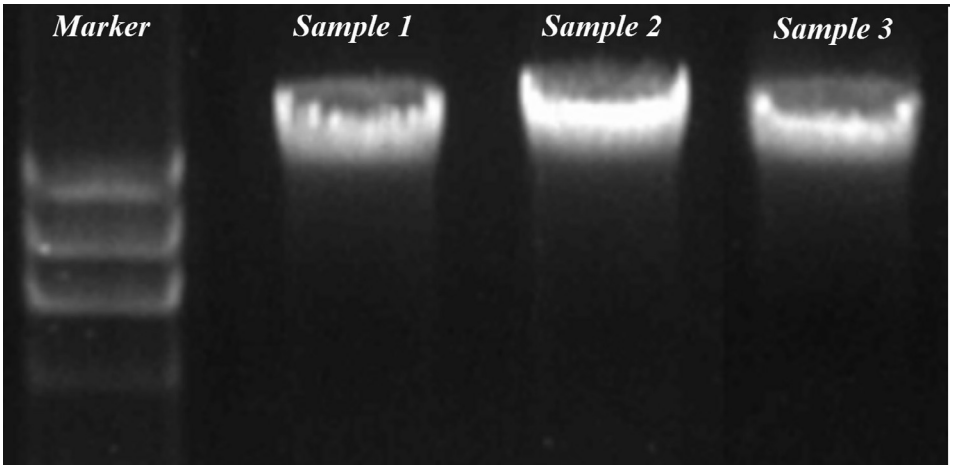

Fig. 1. The whole genomic integrity by agarose gel electrophoresis.

summarized in the Table the sperms mean concentration, volume, vitality, PH, motility and also DNA mean concentration, 260/280 and 260/230 absorbance ratio were determined. The mean ± SD of DNA concentration, A260/280, and A260/230 ratio of all semen samples (N = 50) respectively were 1126.14 ± 810.75, 1.82 ± 0.03 and 1.95 ± 0.16. The integrity of the extracted DNA was assessed by agarose gel electrophoresis (Fig. 1). Gel electrophoresis revealed the high-molecular-weight non-degraded genomic DNA was obtained with this method. The MSP result obtained by agarose gel electrophoresis has been shown in Fig. 2. The sharp bonds revealed the suitable quality of genomic DNA for epigenetic and genetic researches. The Pearson's correlation coefficients calculated between semen parameters (age, sperm concentration, volume, vitality, PH, and motility), DNA concentration and 260/280 and 260/230 absorbance ratio. Correlations between the studied parameters were investigated in 50 semen samples. The degree of correlation was assessed with the coefficient correlation (r) and P-value. In fact, there was no significant correlation between DNA information and the different studied parameters. Interestingly, sperm concentration showed strongly significant correlations with DNA concentration. It was noted that DNA concentration had a positive relationship with an elevated sperm concentration (r = 0.63, P = .001).

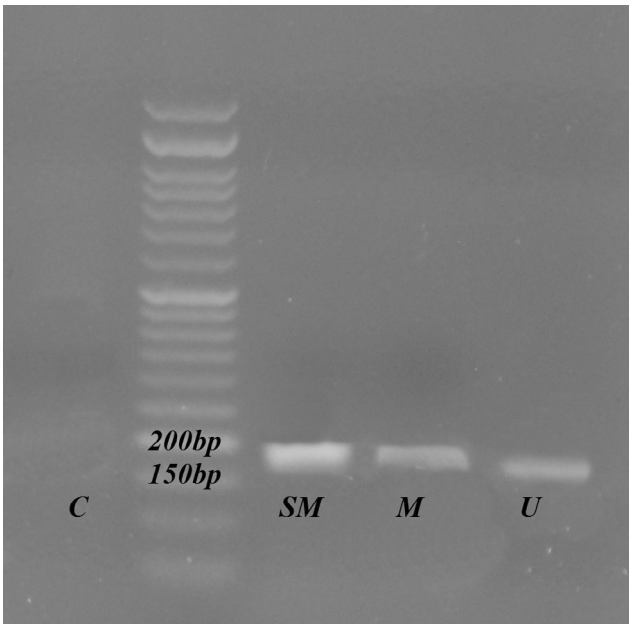

Fig. 2. DNA quality for epigenetic research by MSP assay of Keap1 gene. Primer sets used for amplification were designed as methylated (M) or unmethylated (U). SM; standard methylation, C; control.

4. Discussion and conclusion

The total DNA isolated by this method had appropriate quality for subsequent genetic and epigenetic analysis. In general, DNA requirements for genetic and epigenetic analysis can be met with the use of an expensive extraction kits. Despite many standard protocols reported for DNA extraction and commercial kits, a few of them are useful for complex genomic analysis of sperm cells [6]. As epigenetic and genetic biomarkers assessment become routine in the near future, an economic method is required to extract DNA from sperm and eliminate a large amount of protein combinations and protamins resulting in a high yield of DNA and high integrity. However a few protocols were described before for sperm DNA extraction [7,13,14], our technique was not only very simple but was also economic and reliable. Bahnak in 1988 described a method for isolating mammalian sperm by guanidinium method [13], later some investigators improved guanidinium protocol by adding isopropanol and proteinase K [7,14–17]. Then several companies had employed the modified protocol in expensive kits for isolation of RNA, DNA, and protein [18]. However because this method requires special materials and columns, was not be done routinely in all laboratory. TRIzol (or TRI Reagent) is a mono-phasic reagent composed of phenol and guanidine isothiocyanate and the extraction method include guanidinium thiocyanate-phenol-chloroform [19,20]. After solubilization biological material, the addition of chloroform causes phase separation [19,20]. The TRIzol protocol is widely used for isolating DNA, RNA, and protein from somatic cells [19,20]. It could be more accessible and cheaper than guanidinium protocol. So, changes to the modified protocol by using proteinase K and increasing the incubation time could be used to achieve separation of high molecular weight and quality sperm DNA. The spectrophotometric measurement and the ratio of 260 nm and 280 nm absorbance was used to evaluate the DNA purity [21–26]. Interestingly, this protocol showed, the 260/280 and 260/230 ratios were in optimum and a 260/280 ratio of ~1.8 generally used as a standard for pure DNA [24,27,28]. The 260/230 value was often higher and commonly was ~1.9 or greater [24,27,28]. If the ratio was appreciably lower than expected, it may indicate the presence of contaminants [27,28]. A

low 260/280 nm ratio is indicative of contamination with proteins, [29], and a low 260/230 nm ratio is indicative of contamination with phenol or guanidine carried over during the washing steps. Remaining organic compounds like phenol, guanidine, salt or solvents are also considered inhibitors for downstream applications [30]. Gel electrophoresis revealed non-degraded and suitable quality genomic DNA for epigenetic and genetic analysis. Likewise, the purity and quality of this protocol extracted DNA was closer to the optimum value. So, no contamination and degradation during manual extraction was obvious. Furthermore, the increase in DNA concentration correlated with improved sperm count. It appeared this method may be the best choices to extract high yields of pure and highly concentrated genomic DNA from sperms. Eventually, we concluded this described method was suitable for DNA extraction from human sperm cells to use in genetic and epigenetic studies.

Funding

This project was financially supported by Avicenna Research Institute (Tehran, Iran).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

All authors declare no financial or commercial competing interest.

Consent for publication

Applicable.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Avicenna Research Institute. IR.ACECR.Avicenna.REC.94.9.

Conflict of interest

The authors declared that there is no conflict of interest.

References

[1] A. Zini, A. Agarwal, Sperm Chromatin: Biological and Clinical Applications in Male Infertility and Assisted Reproduction, Springer, New York, 2011.
[2] K. Ni, A.N. Spiess, H.C. Schuppe, K. Steger, The impact of sperm protamine deficiency and sperm DNA damage on human male fertility: a systematic review and meta analysis, Andrology 4 (2016) 789–799.
[3] G.D. Johnson, M. Jodar, R. Pique-Regi, S.A. Krawetz, Nuclease footprints in sperm project past and future chromatin regulatory events, Sci. Rep. 6 (2016).
[4] C.D. Allis, T. Jenuwein, D. Reinberg, Epigenetics, Cold Spring Harbor Laboratory Press, 2007.
[5] U. Schagdarsurengin, A. Paradowska, K. Steger, Analysing the sperm epigenome: roles in early embryogenesis and assisted reproduction, Nat. Rev. Urol. 9 (2012) 609–619.
[6] W. Goodwin, A. Linacre, S. Hadi, An Introduction to Forensic Genetics, John Wiley & Sons, 2007.
[7] L. Barnard, K.I. Aston, Spermatogenesis: Methods and Protocols, Humana Press, 2012.
[8] W.H. Organization, WHO Laboratory Manual for the Examination and Processing of Human Semen, 2010.
[9] J. Tost, I.G. Gut, DNA methylation analysis by pyrosequencing, Nat. Protocols 2 (2007) 2265–2275.
[10] Y. Li, T.O. Tollefsbol, DNA methylation detection: bisulfite genomic sequencing analysis, Epigenet. Protocols (2011) 11–21.
[11] K.E. Schuebel, W. Chen, L. Cope, S.C. Glöckner, H. Suzuki, J.-M. Yi, et al., Comparing the DNA hypermethylome with gene mutations in human colorectal cancer, PLoS Genet. 3 (2007) e157.
[12] P. Mokarram, K. Kumar, H. Brim, F. Naghibalhossaini, M. Saberi-firoozi, M. Nouraie, et al., Distinct high-profile methylated genes in colorectal cancer, PLoS One 4 (2009) e7012.
[13] B. Bahnak, Q. Wu, L. Coulombel, L. Drouet, D. Kerbiriou-Nabias, D. Meyer, A simple and efficient method for isolating high molecular weight DNA from mammalian sperm, Nucl. Acids Res. 16 (1988) 1208.
[14] A. Hossain, B. Rizk, A. Behzadian, I. Thorneycroft, Modified guanidinium thiocyanate method for human sperm DNA isolation, Mol. Hum. Reprod. 3 (1997) 953–956.
[15] H.-F. Yuan, M. Kuete, L. Su, F. Yang, Z.-Y. Hu, B.-Z. Tian, et al., Comparison of three different techniques of human sperm DNA isolation for methylation assay, J. Huazhong Univ. Sci. Technol. Med. Sci. 35 (2015) 938–942.
[16] M.K. De Gannes, Rapid method of processing sperm for nucleic acid extraction, Clin. Res. (2014).
[17] S.-H. Kim, T. Hamada, Rapid and reliable method of extracting DNA and RNA from sweetpotato, Ipomoea batatas (L) Lam, Biotechnology letters. 27 (2005) 1841–1845.
[18] H. Wu, M.K. de Gannes, G. Luchetti, J.R. Pilsner, Rapid method for the isolation of mammalian sperm DNA, BioTechniques 58 (2014) 293.
[19] A.B. Hummon, S.R. Lim, M.J. Difilippantonio, T. Ried, Isolation and solubilization of proteins after TRIzol® extraction of RNA and DNA from patient material following prolonged storage, BioTechniques 42 (2007) 467.
[20] D.C. Rio, M. Ares, G.J. Hannon, T.W. Nilsen, Purification of RNA using TRIzol (TRI reagent), Cold Spring Harbor Protocols 2010 (2010).
[21] W.-J. Kong, Y. Wang, Q. Wang, Y.-C. Han, Y.-J. Hu, Comparison of three methods for isolation of nucleic acids from membranate inner ear tissue of rats, Chin. Med. J. 119 (2006) 986–990.
[22] G.A. Fahle, S.H. Fischer, Comparison of six commercial DNA extraction kits for recovery of cytomegalovirus DNA from spiked human specimens, J. Clin. Microbiol. 38 (2000) 3860–3863.
[23] K. Riemann, M. Adamzik, S. Frauenrath, R. Egensperger, K.W. Schmid, N.H. Brockmeyer, et al., Comparison of manual and automated nucleic acid extraction from whole-blood samples, J. Clin. Lab. Anal. 21 (2007) 244–248.
[24] K. Riemann, M. Adamzik, S. Frauenrath, R. Egensperger, K.W. Schmid, N.H. Brockmeyer, et al., Comparison of manual and automated nucleic acid extraction from whole blood samples, J. Clin. Lab. Anal. 21 (2007) 244–248.
[25] L.A. Dauphin, R.E. Walker, J.M. Petersen, M.D. Bowen, Comparative evaluation of automated and manual commercial DNA extraction methods for detection of Francisella tularensis DNA from suspensions and spiked swabs by real-time polymerase chain reaction, Diagnos. Microbiol. Infect. Dis. 70 (2011) 299–306.
[26] A. Psifidi, C.I. Dovas, G. Banos, A comparison of six methods for genomic DNA extraction suitable for PCR-based genotyping applications using ovine milk samples, Mol. Cell. Probes 24 (2010) 93–98.
[27] P. Towner, Purification of DNA, Essent. Mol. Biol. 1 (1991) 47–68.
[28] L.M. Schiebelhut, S.S. Abboud, L.E. Gómez Daglio, H.F. Swift, M.N. Dawson, A comparison of DNA extraction methods for high throughput DNA analyses, Mol. Ecol. Resour. 17 (2016) 721–729.
[29] I.G. Wilson, Inhibition and facilitation of nucleic acid amplification, Appl. Environ. Microbiol. 63 (1997) 3741.
[30] A. Salonen, J. Nikkilä, J. Jalanka-Tuovinen, O. Immonen, M. Rajilić-Stojanović, R. A. Kekkonen, et al., Comparative analysis of fecal DNA extraction methods with phylogenetic microarray: effective recovery of bacterial and archaeal DNA using mechanical cell lysis, J. Microbiol. Methods 81 (2010) 127–134.