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Fourier Transform Infrared Spectroscopy studies of Human Semen Nucleic acid

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ABSTRACT

Sperms storage through freezing has been useful for men who have difficulty masturbating during assisted reproductive technology programs and before impotency caused by chemotherapy, vasectomy, and other procedures. Studies were undertaken to assess the extent of cryoinjury to sperm after repeated freezing and thawing. Fourier transform infrared (FTIR) spectra of human semen DNA have been investigated in the 700–4000 cm^{-1} region. The obtained results for the FTIR spectra represent the changes in the structure of DNA, which seem to be connected with modification of bases and sugars, and redistribution of the H-bond network. Our findings showed that the FTIR technique can distinguish between different DNA samples of biological interests. Furthermore, this study gives support to the idea that a measurable degree of damage to the DNA could occur during the preservation time in liquid nitrogen.

Key words: *FTIR-ATR, DNA damage, IR Absorbance, Sperms.*

INTRODUCTION

In general, the fertility rate of women is largely depends on the male factors. A number of studies based on many mammalian species demonstrate that the male factors caused infertility in the female due to many problems, *e.g.* congenital abnormalities, gene mutation, infectious disease and the damage or fragmentation of DNA of the spermatozoa [1, 2]. During the last decade, one area of research interest is the studies on the sperm DNA damage that influence male fertility in humans or animals [3]. Earlier studies demonstrated that the sperm DNA can be damaged by various mechanisms such as reactive oxygen species (ROS) and apoptosis [1, 2]. It has been revealed that the DNA damage of the human an animal spermatozoa depends on two main factors: tight packaging of chromatin of the spermatozoa and the amount of antioxidant substances in seminal plasma [4].

There are several methods to determine the sperm DNA damage including, for instance, the sperm chromatin structure assay (SCSA) [5], Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) [6], comet assay [7], and acridine orange (AO) staining [8]. AO staining is commonly used for assessing sperm DNA damage in many mammalian species including humans, dogs, bulls and cats [9]. Using this technique, the DNA damage of the frozen-thawed sperm was 48.1% in humans [10]. Development of research methods provides opportunities for monitoring biomedical changes and metabolic processes with the use of various spectroscopic techniques. At present, there are a lot of spectroscopic methods based on various effects of interactions between radiation and matter, among which vibrational spectroscopy deserves special attention such as FTIR. The objective of this study was to investigate the effect of storage of human semen in liquid nitrogen on sperm DNA.

MATERIALS AND METHODS

The semen DNA samples were obtained from infertile Libyan patients. The A_{260}/A_{280} ratio was 1.85, showing that the DNA was sufficiently free from protein. The UV absorbance at 260 nm of the human semen DNA was 0.32 (path length was 1 cm) and the final concentration of the DNA solution was 2.0 micromolar dissolved in TRIS-EDTA (TE) buffer. Infrared spectra were recorded on a Varian 610/660-IR FT-IR spectrometer equipped with nitrogen cooled HgCdTe detector and KBr beam splitter. The solution spectra are taken using AgBr windows with resolution of 2 to 4 cm^{-1} and 100-500 scans. Each set of infrared spectra was taken (three measurements) on three identical samples with the same DNA concentrations. The water subtraction was carried out with TE solution used as a reference. A good water subtraction is achieved as shown by a flat baseline around 2200 cm^{-1}, where the water combination mode is located. This method is a rough estimate, but removes the water content in a satisfactory way [11]. The difference spectra (semen DNA before and after nitrogen freezing) are produced, using a sharp DNA band at 1040 cm^{-1}. These bands, due to the sugar C-C and C-O stretching vibrations exhibit no spectral changes (shifting or intensity variations) on semen DNA before and after freezing and were cancelled upon spectral subtraction. The spectra are smoothed with a Savitzky-Golay procedure [11]. The intensity ratios of several DNA in-plane vibrations related to A-T and G-C base pairs and the PO_2 stretching are measured.

RESULTS AND DISCUSSION

As reported in the literature the success of cryopreservation depends upon many other factors, including interactions between cryoprotectant, type of extender, cooling rate, thawing rate and packaging, as well as the individual human variation [12]. Some changes for sperm DNA during the cryopreservation process is unavoidable due to the processing procedures prior to freezing as well as during the actual freezing process. Moreover, motility of spermatozoa has proven to be an even poorer indicator of fertility in frozen–thawed samples [13, 14]. Regardless of all these considerations, for cryopreservation to be considered a success the process should enable a spematozoon to keep its fertilizing capacity at postthaw. To achieve this it must retain its ability to produce energy *via* metabolism; to maintain normal plasma membrane configuration and integrity; retain its motility; and enzymes, such as acrosin, within the acrosome to allow penetration of the ova. Disruption of any of these functions or abilities will significantly affect the spermatozoon's ability to achieve fertilization. The greatest risk to the maintenance of these attributes is presented by the formation of ice crystals and the resultant movement of water up osmotic gradients during the process of cryopreservation.

The FTIR spectra of DNA show many characteristic bands, which are sensitive to denaturation, dehydration and conformational transition. The FTIR spectrum in **Fig. 1** can be divided into four characteristic spectral ranges: base, base-sugar, sugar-phosphate and sugar vibrations. Within the 1800–1500 cm^{-1} region, there are absorption bands associated with C=O, C=N, C=C stretching vibrations and N–H bending vibrations of bases. These bands are sensitive to

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changes in the base stacking and base pairing interactions. Bands occurring in the interval 1500-1250 cm^{-1} are assigned to vibrations of the bases and base-sugar connections. The range of 1250–1000 cm^{-1} refers to vibrations of a phosphate sugar chain. The bands within this range, including PO^{-}_{2} symmetric and asymmetric stretching vibrations as well as C–O stretching vibrations of the side chain, show high sensitivity to conformational changes of the backbone. Within the last region (1000–800 cm^{-1}), there are absorption bands associated with sugar vibrations, which correlate with the various nucleic acid sugar puckering modes (C2′-endo and C3′-endo) [15-17].

In order to characterize the human semen DNA, the infrared spectra of DNA solutions were recorded using constant amount of DNA and the findings are presented in Figure 1. The DNA in-plane vibrations at 1750-1500 cm^{-1} related to the G-C and A-T base pairs and the back bone phosphate group at 1250-1000 cm^{-1} [15-17]. The mainly guanine carbonyl vibration at 1527cm^{-1} of the semen DNA before nitrogen freezing decreased in intensity and shifted towards a higher frequency at 1550 cm^{-1} upon nitrogen freezing (**Fig. 1**). Similarly for semen DNA before nitrogen freezing a decrease in the intensity of the backbone PO_2 asymmetric stretching band at 1037 cm^{-1} was observed, which shifted towards a higher frequency at 1064 cm^{-1} for the semen DNA after freezing (**Fig. 1**). Further evidence regarding the effect of nitrogen on the semen DNA comes from a major shifting at 2944 for the semen DNA before freezing to the 2923 cm^{-1} for the semen DNA after freezing. The observed spectral changes may be due to the participation of the G-C bases (mainly guanine) and the backbone PO_2 group in the semen DNA before and after nitrogen freezing. Therefore, it can be assumed that the nitrogen freezing has effect mainly on G-C bases and the backbone phosphate groups of the DNA.

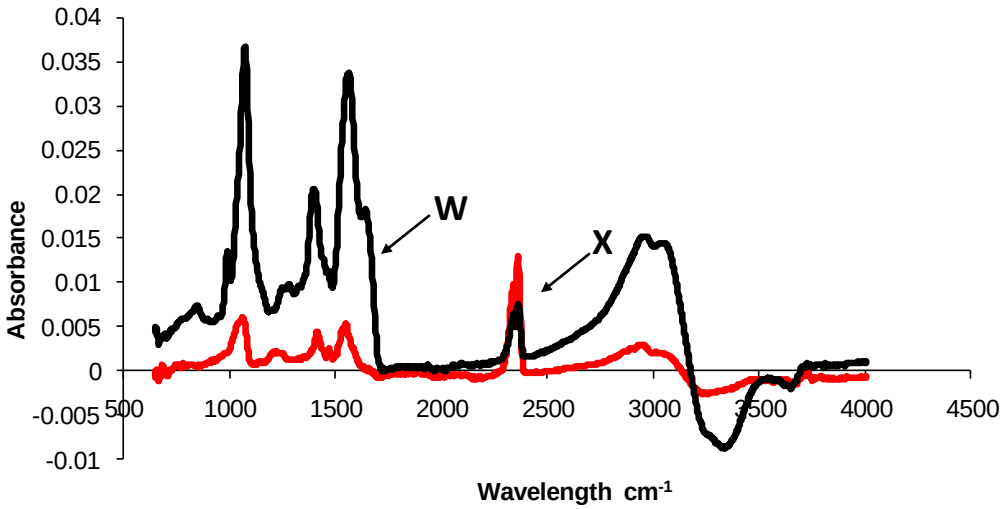

Fig. 1: FTIR spectra of (X) human semen DNA after freezing (W) human semen DNA before freezing in TE buffer at physiological pH

Infrared spectroscopy has proven to be a perfect tool for investigating the effects of hydration levels on the DNA structure [15, 18]. The consequence of DNA dehydration from 95% to 88% relative humidity is its transition from the B-type into the A-DNA. The process is accompanied by infrared spectral changes. The B-DNA (**Fig. 1, W**) is characterized by the presence of 835, 1088, 1223 and 1715 cm^{-1} marker bands. In the A-type of 88% relative humidity, the bands (**Fig. 1, X**) are shifted to 861, 1089, 1234 and 1709 cm^{-1}, respectively. Moreover, the A-DNA infrared spectrum contains a new band at 1185 cm^{-1} assigned to the vibrations of deoxyribose in the C3′-endo conformation [19]. Further dehydration of a DNA molecule leads to the loss of its ordered structure, which is manifested by decreased intensities of bands in the 1709-1715 cm^{-1} region. In DNA samples with the water content of 7.2, 3.3 and 1.2 water molecule per nucleotide (wpn), the band in this region disappears, which indicates the loss of interactions between bases and partial DNA denaturation [15, 18]. DNA dehydration results in changes within other infrared bands. Due to dehydration, strong shifts of bands corresponding to the PO^{-}_{2} asymmetric stretching vibrations from 1220 cm^{-1} (95% RH) to 1240 cm^{-1} (0% RH), PO^{-}_{2} symmetric stretching vibrations from 1089 cm^{-1} (95% RH) to 1086 cm^{-1} (0% RH), C–O stretching vibrations of the side chain from 1052 cm^{-1} (95% RH) to 1066 cm^{-1} (0% RH) and C–C stretching vibrations of the backbone from 970 cm^{-1} (95% RH) to 962 cm^{-1} (0% RH) are observed. The changes within these bands prove that dehydration of the macromolecule results in destruction of ordered DNA structure [20, 21].

Finally, the application of frozen-thawed semen technology is currently increasing worldwide. The results obtained in this study have focused on identifying damages during freezing and thawing. Many research centers have made progress in improving survival of frozen-thawed spermatozoa by minimizing the oxidative damage and decreasing the osmotic stress on spermatozoa. Sperms are known to be susceptible to oxidative stress due to their high content of unsaturated fatty acids [22]. In addition to membrane effects, lipid peroxidation can also damage DNA [22]. The addition of antioxidants to extenders has been used as a method to decrease lipid peroxidation and oxidative stress associated with cryopreservation [23]. Different amides, compounds with lower molecular weight than glycerol and penetrate the sperm plasma membrane more readily, have been evaluated as alternative cryoprotectants to glycerol in different animals [24]. These compounds include methyl formamide (MF), dimethyl formamide (DMF) or ethylene glycol (EG) and dimethyl acetamide. They were known to provide greater post-thaw motility when used at different concentrations. Particularly, MF and DMF or EG have been used as alternative cryoprotectants for individual males whose sperm has lesser post-thaw motility when frozen in glycerol [24]. The use of low-density lipoproteins (LDLs), most often isolated from egg-yolk from different species, as additive has proven beneficial for sperm function post-thaw, particularly for DNA-integrity [25]. Attempts to minimize osmotic stress during cryopreservation have included step-wise dilution of cryoprotectants, by incorporating cholesterol-loaded cyclodextrins (CLC) in freezing diluents [26]. As an alternative to adding CLC to extenders provision of polyunsaturated fatty acids in the feed as a means of altering the sperm-lipid membrane profile has been tried with some success in mammals [27].

CONCLUSION

FTIR-ATR technique showed the ability to differentiate between the semen DNA before and after nitrogen freezing. Unlike other techniques, such as fluorescence, radio-labeling, and electrochemical detection, FTIR-ATR does not extra labels and can provide a wealth of information on system. Consequently, FTIR-ATR will have many potential biological applications and is promising for a high-sensitive, label-free, and universal biosensing method in the clinic. The results obtained in the paper indicate that the process of cryopreservation of sperm provide insight and direction into establishing more effective genetic and conservation management programs.

REFERENCES:

1. Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum. Reprod., Update; **2003**: 9(4); 331-345.
2. Carrell DT, De Jonge C, Lamb DJ. The genetics of male infertility: a field of study whose time is now. Arch. Androl., **2006**: 52(4); 269-274.
3. Perez-Llano B, Enciso M, Garcia-Casado P, Sala R, Gosalvez J. Sperm DNA fragmentation in boars is delayed or abolished by

using sperm extenders. Theriogenology, **2006**: 66(9); 2137-2143.
4. De Ambrogi M, Spinaci M, Galeati G, Tamanini C. Viability and DNA fragmentation in differently sorted boar spermatozoa. Theriogenology, **2006**: 66(8); 1994-2000.
5. Evenson DP, Thompson L, Jost L. Flow cytometric evaluation of boar semen by the sperm chromatin structure assay as related to cryopreservation and fertility. Theriogenology, **1994**: 41(3); 637-651.
6. Martins CF, Dode MN, Bao SN, Rumpf R. The use of the acridine orange test and the TUNEL assay to assess the integrity of freeze-dried bovine spermatozoa DNA. Genet. Mol. Res., **2007**: 6(1); 94-104.
7. Fraser L, Strzezek J. The use of comet assay to assess DNA integrity of boar spermatozoa following liquid preservation at 5 degrees C and 16 degrees C. Folia Histochem. Cytobiol., **2004**: 42(1); 49-55.
8. Thuwanut P, Chatdarong K, Techakumphu M, Axner E. The effect of antioxidants on motility, viability, acrosome integrity and DNA integrity of frozen-thawed epididymal cat spermatozoa. Theriogenology, **2008**: 70(2); 233-240.
9. Thuwanut P, Chatdarong K, Techakumphu M, Axner E. The effect of antioxidants on motility, viability, acrosome integrity and DNA integrity of frozen-thawed epididymal cat spermatozoa. Theriogenology, **2008**: 70(2); 233-240.
10. Hammadeh ME, Szarvasy D, Zeginiadou T, Rosenbaum P, Georg T, Schmidt W. Evaluation of cryoinjury of spermatozoa after slow (programmed biological freezer) or rapid (liquid nitrogen vapour) freeze-thawing techniques. J. Assist. Reprod. Genet., **2001**: 18(7); 364-370.
11. Jackson M., Mantsch H.H. FTIR spectroscopy in the clinical sciences. Biomedical Application of Spectroscopy Advances in Spectroscopy. Chichester, UK: Wiley&Sons, **1996**; 185-215.
12. Cotter PZ, Goolsby HA, Prien SD. Preliminary evaluation of a unique freezing technology for bovine spermatozoa cryopreservation. Reprod. Domest. Anim., **2005**: 40(2); 98-99.
13. Samper JC, Hellander JC, Crabo BG. Relationship between the fertility of fresh and frozen stallion semen and semen quality. J. Reprod. Fertil., Suppl; **1991**: 44; 107-114.
14. Sieme H, Katila T, Klug E. Effect of semen collection practices on sperm characteristics before and after storage and on fertility of stallions. Theriogenology, **2004**: 61(4); 769-784.
15. Guzman MR, Liquier J, Taillandier E. Hydration and conformational transitions in DNA, RNA, and mixed DNA-RNA triplexes studied by gravimetry and FTIR spectroscopy. J. Biomol. Struct. Dyn., **2005**: 23(3); 331-339.
16. Geinguenaud F, Liquier J, Brevnov MG, Petrauskene OV, Alexeev YI, Gromova ES, Taillandier E. Parallel self-associated structures formed by T,C-rich sequences at acidic pH. Biochemistry, **2000**: 39(41); 12650-12658.
17. Dagneaux C, Liquier J, Taillandier E. Sugar conformations in DNA and RNA-DNA triple helices determined by FTIR spectroscopy: role of backbone composition. Biochemistry, **1995**: 34(51); 16618-16623.
18. Guzman MR, Liquier J, Brahmachari SK, Taillandier E. Characterization of parallel and antiparallel G-tetraplex structures by vibrational spectroscopy. Spectrochim Acta A Mol. Biomol. Spectrosc., **2006**: 64(2); 495-503.
19. Whelan DR, Bambery KR, Heraud P, Tobin MJ, Diem M, McNaughton D, Wood BR. Monitoring the reversible B to A-like transition of DNA in eukaryotic cells using Fourier transform infrared spectroscopy. Nucleic Acids Res., **2011**: 39(13); 5439-5448.
20. Pichler A, Rauch C, Flader W, Wellenzohn B, Liedl KR, Hallbrucker A, Mayer E. The conformer substates of nonoriented B-type DNA in double helical poly(dG-dC). J. Biomol. Struct. Dyn., **2003**: 20(4); 547-559.
21. Keller PB, Loprete DM, Hartman KA. Structural forms and transitions of poly(dG-dC) with Cd(II), Ag(I) and NaNO3. J. Biomol. Struct. Dyn., **1988**: 5(6); 1221-1229.
22. Clulow JR, Mansfield LJ, Morris LH, Evans G, Maxwell WM. A comparison between freezing methods for the cryopreservation of stallion spermatozoa. Anim. Reprod. Sci., **2008**: 108(3-4); 298-308.
23. Bilodeau JF, Blanchette S, Gagnon C, Sirard MA. Thiols prevent H2O2-mediated loss of sperm motility in cryopreserved bull semen. Theriogenology, **2001**: 56(2); 275-286.
24. Bianchi I, Calderam K, Maschio EF, Madeira EM, da Rosa UR, Corcini CD, Bongalhardo DC, Correa EK, Lucia T, Jr., Deschamps JC, Correa MN. Evaluation of amides and centrifugation temperature in boar semen cryopreservation. Theriogenology, **2008**: 69(5); 632-638.
25. Bwanga CO. Cryopreservation of boar semen. I: A literature review. Acta Vet. Scand., **1991**: 32(4); 431-453.
26. Wessel MT, Ball BA. Step-wise dilution for removal of glycerol from fresh and cryopreserved equine spermatozoa. Anim. Reprod. Sci., **2004**: 84(1-2); 147-156.
27. Purdy PH, Graham JK. Effect of cholesterol-loaded cyclodextrin on the cryosurvival of bull sperm. Cryobiology, **2004**: 48(1); 36-45.

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