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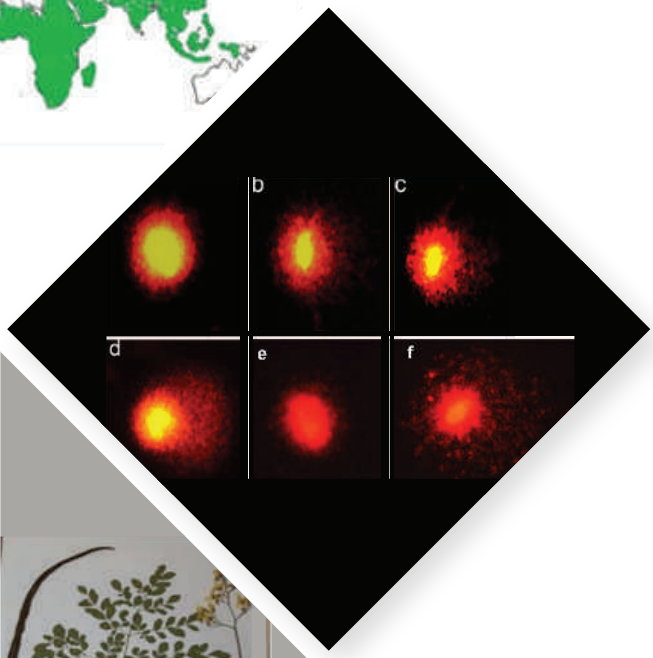

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Hospital & Research Institute

"Chettinad International Fertility Colloquium"

on

Male and Female Reproduction

30.09.2018 Sunday between 7.00 am and 4.00 pm

at

Mini Auditorium, Chettinad Health City

Department of Andrology and Reproductive Medicine, Chettinad Hospital & Research Institute,

cordially invites you for

"Chettinad International Fertility Colloquium 2018"

Eminent and experienced speakers from India and abroad will be addressing various issues in male and female reproduction - Dr. Sathanandhan (UK), Prof.Dr.N.Pandiyan (USA), Dr. Devika Gunasheela (Bengaluru), Dr. Rajvi Mehta (Mumbai), Dr. Sujatha (Chennai) and many more speakers.

Original articles related to male and female reproduction and infertility are invited for free paper session. Selected best papers will be awarded prizes during the proceedings of the conference.

Round table breakfast/Lunch session: Programme of round table is subject to availability of faculty & registrants. Needs prior registration and limited to 10/ table.

Provisional topics are: 1. Male infertility 2. Female infertility 3. Embryology

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Chettinad Health City

MEDICAL JOURNAL

Contents

Editorial

Greetings from the Editorial Board of Chettinad Health City Medical Journal!

Advances in early cancer diagnosis and treatment regimens have significantly improved survival rates, allowing patients and professionals to think beyond the cure for cancer to the future quality of life. Fertility impairment is a significant concern for many pediatric, adolescent and young adult cancer survivors. Treatment modalities such as chemotherapy, radiotherapy and surgery can impair the future fertility of men, women and children combating with cancer. Options to preserve the fertility of these patients holds significant value in improving the quality of their life post treatment. Though the field of fertility preservation has started gaining attention just a decade ago, there is a serious lack of collaborative effort between cancer care and fertility management.

This special issue of Chettinad Health City Medical Journalis a compilation of the contributions of all the well-known researchers and clinicians who are working in the field of fertility preservation.An original article aims to understand the damaging effects of radiation on spermatozoa of men exposed to therapeutic or accidental exposures to radiation.

Native plants of India are known for their medicinal values and other diverse applications. One such plant is Moringa oleifera better known as the drumstick tree, appears to also have a role in male fertility preservation, as described in a review article. Another review highlights the importance of cryopreservation in infertility management and fertility preservation.

A seminar article educates the readers on the different options available in fertility preservation for cancer patients, along with providing a perspective on future strategies. Having understood the different options, it is essential to also be aware of the social and ethical issues surrounding oncofertility, which are discussed through another article.

Hope you enjoy the informative articles. Look forward to your opinions and feedback!

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Original Article

Paternal Age Influences Human Sperm Chromatin Sensitivity to Ionizing Radiation

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Chettinad Health City Medical Journal 2018; 7(3): 95 - 99

Abstract

Objective : To understand the age related sensitivity of human and mouse sperm chromatin to radiation.

Study design: This prospective study examined the comparative *in vitro* radiosensitivity of sperm DNA from normozoospermic human ejaculate (N= 47) and mouse epididymal spermatozoa (N= 40), from various age groups. Human ejaculates and mouse sperm suspension were exposed to 0 and 5 Gy,-radiation using ^{60}Cobalt tele-therapy unit and sperm DNA damage was assessed by single cell gel electrophoresis (neutral comet assay).

Results: The DNA fragmentation analysis of 5 Gy irradiated spermatozoa has revealed a significant increase in olive tail moment (OTM) in men above 40 years ($P<0.05$). In contrast, the OTM did not vary in mouse spermatozoa in any of the irradiated groups.

Conclusion: Though, the data has emerged from in vitro radiation exposure, from the clinical point of view, the study warns that men who are above 40 years are at higher risk of acquiring sperm DNA fragmentation after therapeutic and accidental radiation exposures.

Key words: anti-cancer agents, comet assay, ionizing radiation, paternal age, sperm DNA integrity.

Introduction

It is now evident that the anticancer treatments such as radiation and chemotherapeutic agents pose a great threat to sperm chromatin integrity.[1,2] Though, DNA repair mechanisms play a pivotal role in mutagenesis, certain repair pathways aberrantly function with the advancement in age.[3,4] It has been shown that mutagenic response to ionizing radiation in germline is modulated by age.[5] Importantly, the persistence of unrepaired DNA lesions in germ cells may have deleterious consequences of transgenerational genomic instability thereby resulting in abnormal reproductive outcome in the offspring.[6-9]

Sperm chromatin is composed of tightly packed DNA with small basic proteins called protamines, which helps in chromatin remodeling process.[10] It is now evident that altered protamine level increases the susceptibility of sperm to undergo DNA fragmentation and eventual poor outcomes in assisted reproduction.[11] The proportion of protamine and nucleosomal structure are different in mouse and human spermatozoa[10-12] in which human sperm nuclear DNA has a heterogeneous structure with some regions and genes remaining associated with histones or with other proteins.[10,13,14] Hence this difference in chromatin structure may demonstrate the differences in sperm DNA damage susceptibility to a genotoxic agent.[15,16]

Although there has been a drastic increase in the incidence of cancer in men, the advancements in therapeutic regimes have significantly improved the survival of the affected individuals. The number of survivors in the reproductive age group is increasing; hence it is important to evaluate the sensitivity of sperm chromatin structure to anticancer agents. The current scenario demands in depth understanding of the differential sensitivity of spermatozoa with respect to the age of the individuals. In this study we investigated the spermatozoal susceptibility to ionizing radiation in vitro. To examine the comparative radiosensitivity of sperm DNA, both mouse and human spermatozoa from various age groups were included in this study.

Materials and methods

Human subjects

This prospective study included 47 men attending University infertility clinic for semen evaluation. Only normozoospermic ejaculates were included in this study as poor quality ejaculates are known to carry higher level of baseline DNA fragmentation.[17] The study was approved by the Institutional Ethical Committee.

Semen samples were obtained between 3-5 days of sexual abstinence by masturbation in sterile nontoxic containers. Semen analysis was performed within one hour of collection under sterile conditions. Upon completion of liquefaction, the sample was mixed well and evaluated for physical and microscopic characteristics according to WHO criteria.[18]

Animals

The animal care and handling were done according to the institutional guidelines for animal experimentation and the proposal was approved by the Institutional Ethical Committee. Healthy Swiss albino male mice between the age of 8-52 weeks maintained under the controlled conditions of temperature (23±2°C) and light (12 h light/dark cycles) with standard diet and water ad libitum were used in this study. The animals were sacrificed and spermatozoa were extracted from the caudae epididymis in 2 mL of pre-warmed Earle's Balanced Salt Solution (EBSS) (Medium 199,Sigma Chemical Co., St. Louis, USA Cat. No. M5017) supplemented with 0.1% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, USA Cat. No. A3311). The spermatozoa were centrifuged and the pellet was resuspended in 500 µL of medium for the further experiments

In vitro irradiation

Fixed volume 500 µL of the human ejaculate and mouse sperm suspension were transferred to a petridish and then exposed to 0 and 5 Gy-radiation using ^{60}Co tele-therapy unit with a dose of 1 Gy/ min at room temperature.

Single cell gel electrophoresis (Neutral comet assay)

Within 30 mins after irradiation, the DNA fragmentation in spermatozoa was assessed by the comet assay as described earlier[19] with minor modifications. Briefly, the spermatozoa suspended in sterile Phosphate Buffer Saline (PBS), (pH 7.4) were mixed with equal volume of 1 % low melting point (LMP) agarose (Sisco Research Laboratories, India, Cat. No. 0140151) and layered on a slide pre-coated with 1 % normal melting point (NMP) agarose (Sisco Research Laboratories, India, Cat.No.0144162). Agarose was allowed to gel by placing the slides at -4°Cfor 5 mins. A third coat of agarose was layered over the second layer followed by overnight incubation in lysing solution (2.5 M sodium chloride, 100 mM disodium EDTA, 10 mMTrizma base, 10% DMSO, 1 % Triton X-100, 20 mM DTT (pH 10) at 4°C. Sperm DNA unwinding was carried out by immersing the slides in electrophoresis buffer (300 mM Sodium acetate, 100 mMTris base, pH= 9) for 20 mins followed by electrophoresis at 20 V (VcM= 0.74 V/cm, 100 mA) for 60 mins (performed at 4°C). The slides were neutralized by flooding with neutralization buffer (0.4 M TrisHCl buffer) for 5 minutes, dehydrated by immersing them in chilled absolute alcohol for 10 mins and stored till further processing.

For visualization and scoring, the slides were rehydrated in cold PBS for 10 minutes and stained with 2g/ml Ethidium bromide. The slides were observed under a fluorescent microscope (Imager-A1, Zeiss, Germany) and images were captured using 40 X objective. A minimum of 50 randomly selected images were captured from each slide which was coded to prevent observer's bias. The sperm with DNA damage attained the shape of comet with tail region consisting of fragmented DNA and head region with intact DNA. The representative images have been provided in Supplementary Fig 1. The comet evaluation of the captured images was done using Kinetic Imaging software (Komet 5.5, UK).

Statistical analysis

The data represents mean and standard error (Mean ± SEM) of the values. The statistical significance level was calculated using One Way Analysis of Number of Variance (ANOVA) (Tukey test) using Graph PADInstat software, USA. The graphs were plotted using Origin 6.0 (USA).

Results

Human study

The present study included a total of 47 normozoospermic men from various age groups (Group I, 21-30 years, N=10; Group II, 31-40 years, N=28; Group III, 41-50 years, N=9). The sperm count per milliliter of ejaculate (mean ± SEM) in Group I, II and III was 62.0 ± 10.72; 49.25±5.54 and 35.25±5.92 respectively which was not significantly different between each group. Similarly, total sperm motility also did not show significant difference between any of the groups (Group I: 65.22 ± 5.21, Group II: 63.25 ± 1.68; Group III: 56.37 ± 4.55). Neutral comet assay was performed to determine the extent of DNA fragmentation in the ejaculates of various groups. The DNA fragmentation as measured by OTM did not vary between group I & II. Though, a moderate increase in the baseline OTM was observed in group III, the difference was not statistically significant with other groups studied. Exposure to 5 Gy-radiation induced a marginal, non-significantly higher level of OTM in group I and II within 30 min after irradiation. However, the OTM was increased by approximately 1.7 folds in group III which was significantly higher than the unirradiated control (P<0.05) (Fig 1).

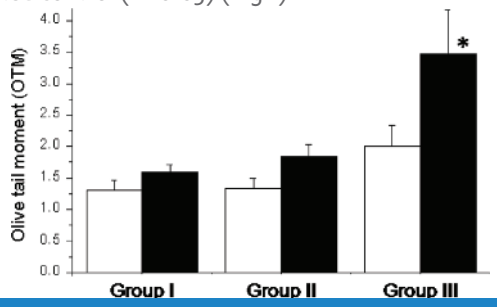
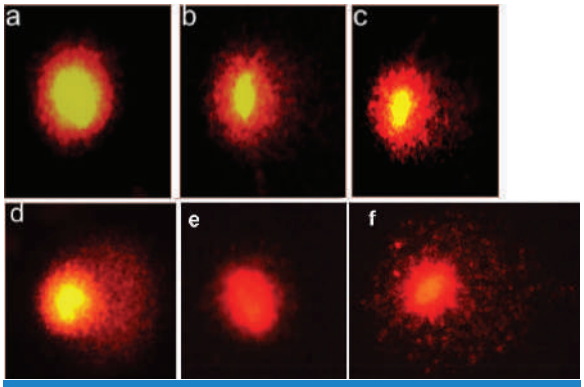


Fig 1: DNA fragmentation in in vitro irradiated human spermatozoa from various age groups. Olive tail moment in unirradiated control and 5 Gy-irradiated human spermatozoa(*P < .05 Vs unirradiated).



Supplementary Fig 1 : Representative images of single cell gel electrophoresis (Comet) a-d) human sperm cells displaying various degrees of DNA fragmentation. e-f) sperm cells from mice showing various degrees of DNA fragmentation.

Mouse study

In order to determine the species specific sensitivity of ionizing radiation in relation to the age, mouse spermatozoa from 8 (Group I), 12(Group II), 26 (Group III), and 52 (Group IV), weeks were used in replicates.[20] The number of animals (N) used in each group was 10. Similar to human ejaculates, the sperm count and motility in mice did not vary significantly between each group (sperm count in millions/ml: 18.08 ± 2.8, 17.13 ± 2.1, 16.41 ± 2.5, 16.61 ± 3.4 and percent sperm motility: 66.9 ± 2.19, 51.9 ± 6.09, 53.2 ± 5.8, 58.4 ± 5.7 in 8, 12, 26, 52 weeks groups respectively). The DNA fragmentation was quantified in at least 50 spermatozoa from each group. The baseline OTM showed a minimum non-significant variation between different age groups studied. *In vitro* -irradiation was performed on mouse spermatozoa and conditions were kept identical to human sperm irradiation. In contrast to human spermatozoa, the OTM level did not vary in any of the irradiated group including the highest age group of 52 weeks which demonstrated only 1.14 fold non significant increases in the OTM level when compared to unirradiated control (Fig 2).

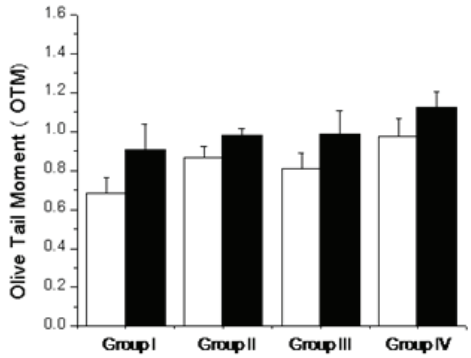


Fig 2: DNA fragmentation in in vitro irradiated mouse spermatozoa from various age groups. Olive tail moment in unirradiatedcontrol and 5 Gy-irradiated mouse spermatozoa.

Discussion

In this study, we compared the sperm chromatin sensitivity to in vitro exposed ionizing radiation from the individuals of varying age group. In vitro irradiation of spermatozoa enabled us to elucidate the direct and untargeted effect of radiation on male germ cells in relation to the age of the individuals. Parallely, mouse spermatozoa were also evaluated to compare the species specific response to ionizing radiation using neutral comet assay. We found that human spermatozoa from the subsets of age between 40-50 years had increased sensitivity to radiation induced DNA fragmentation. In contrast, spermatozoa from mouse up to 52 weeks of age failed to show any significant increase in the radiation induced DNA damage.

Ageing is associated with an overall increase in oxidative stress possibly due to decreased ATP and increased reactive oxygen species (ROS) production.[21] Studies have shown that the effect of age on human spermatozoa is associated with change in motility pattern[22] and DNA integrity[17,23] which are probably due to the compromised maturing efficiency of spermatozoa in older mammals during their epididymal transit.[24] However, we did seemarginal increase in the baseline DNA fragmentation both in mice and human spermatozoa, though studies have shown different protamine and nucleosomal structure in mice and human[10,12] which are responsible for compactness of sperm nuclear DNA. Along with difference in sperm nuclear structure, study subjects were not uniformly distributed in human study; because of this reason may be we were not able to prove statistical difference in baseline DNA damage. Hence, giving the contradictory results to the current literature.

It has been shown that oxidative stress constitutes one of the mechanisms for inducing the DNA damage in spermatozoa, and defective chromatin remodeling renders spermatozoa susceptible to oxidative attack.[25] To investigate whether sperm with dissimilar chromatin architecture exhibit differential sensitivity to genotoxic insult, in this study, both human and mouse spermatozoa from different age groups were exposed to -radiation. The results of our study showed that the direct effect of ionizing radiation on sperm is dependent on the age in human but not in mouse model.

The gonadotoxic effects of cancer therapy especially on sperm DNA integrity is well documented[26,27], yet it remains controversial whether the susceptibility of spermatozoa to commonly used ionizing radiation is related to the age of the patients. In order to test this hypothesis, we included 47 normozoospermic ejaculates from different age groups exposed to radiation. Though, we did see marginal increase in sperm DNA fragmentation until 40 years of age, men who are above 40 years of age had significantly elevated level of sperm DNA fragmentation in response to radiation. The increased genetic abnormalities with age have largely been attributed to genetic changes in the egg and sperm

of the parents.[28] There are reports that DNA damage and chromosomal errors increase with age in sperm[17,29] possibly due to qualitative change in the lineage of sperm producing cells as they age and/or due to defective DNA repair machinery.[28] Since mature sperm is transcriptionally silent, the elevated DNA lesions observed in the present study is unlikely due to aberrant repair pathway but possibly because of age related changes in chromatin architecture. The results also emphasize on the fact that the men who are above 40 years are more prone to acquire sperm DNA damage.

Apart from the differences in the proportion of protamine and nucleosomal structure in mouse and human spermatozoa[10,12,] there were also some differences in the resistance to oxidation between the sperm nuclei of the different species.[30] It has been shown that both mouse and human spermatozoa were quite resistant to in vitro irradiation.[31] However, the distribution of DNA damage among individual sperm cells after irradiation in mice was heterogeneous.[32] In contrast, the human sperm is most susceptible to undergo DNA fragmentation in response to in vitro stimulation when compared to mouse and bull sperm.[16] Our results suggest that sensitivity of mouse spermatozoa to in vitro genotoxic insult is much lower than that of human spermatozoa which agrees with earlier report.[16] The proportion of protamine 2 is 67% and 34% in human and mouse spermatozoa respectively. In addition, some nucleosomal structure is retained in human (up to 15%) and, in smaller quantity(~1%) in mouse spermatozoa[10,12], which could explain the resistance of mouse sperm chromatin to undergo fragmentation in response to in vitro irradiationin comparison to human sperm.

There are limitations in comparing age related sensitivity between two species as mice and humans have a maximum life-span of 4 years and 120 years, respectively which are at two extremes of the longevity range.[33] In spite of their similarities at the molecular, cellular and physiological level, there are dissimilarities in the rates at which they age.[34] However, mouse models are still considered as one of the primary systems in studies of human longevity. Hence, mice model was used in the present study to compare the in vitro radiosensitivity of human spermatozoa of various age groups.

Despite of several observed age related changes, our results demonstrate that irradiation of human spermatozoa results in age dependent increase in the level of DNA fragmentation possibly due to age related differences in chromatin architecture. Therefore,from the clinical point of view, the study demonstrates that men who are above 40 years are at higher risk of acquiring sperm DNA fragmentation after therapeutic and accidental radiation exposures hence proper counseling should be seriously considered as sperm chromatin integrity is of fundamental importance for these subjects to father a child. As the present study is restricted to in vitro irradiation of spermatozoa, further studies are required to confirm our observation in patients who have received radiation therapy.

References

1) Spermon JR, Ramos L, Wetzels AM,Sweep CG, Braat DD, Kiemeney LA.et alSperm integrity pre- and post-chemotherapy in men with testicular germ cell cancer. Hum Reprod 2006; 21(7):1781-6.

2) Pacey AA. Environmental and lifestyle factors associated with sperm DNA damage. Hum Fertil (Camb). 2010; 13(4):189-93.

3) Intano GW, McMahan CA, McCarrey JR,Ronald BW, Allison EM,Yoshihiro M et al. Base excision repair is limited by different proteins in male germ cell nuclear extracts prepared from young and old mice. Mol Cell Biol.2002; 22(7):2410-8.

4) Xu G, Spivak G, Mitchell DL,Mori T, McCarrey JR, McMahan CA et al. Nucleotide excision repair activity varies among murinespermatogenic cell types.Biol Reprod.2005; 73(1):123-30.

5) Xu G, McMahan CA, Hildreth K, Garcia RA, Herbert DC, Walter CA. Ionizing radiation-induced mutant frequencies increase transiently in male germ cells of older mice. Mutat Res.2012; 744:135-9.

6) Toyoshima M, Shimura T, Adiga SK, Taga M, Shiraishi K, Inoue Met al. Transcription-independent suppression of DNA synthesis by p53 in sperm-irradiated mouse zygotes. Oncogene. 2005; 24(20):3229-35.

7) Adiga SK, Toyoshima M, Shiraishi K, Shimura T, Takeda J, Taga Met al. p21 provides stage specific DNA damage control to preimplantation embryos.Oncogene.2007; 26(42):6141-9.

8) Adiga SK, Upadhya D, Kalthur G, Bola Sadashiva SR, Kumar P. Transgenerational changes in somatic and germ line genetic integrity of first-generation offspring derived from the DNA damaged sperm.Fertil Steril.2010; 93(8):2486-90.

9) Kumar D, Upadhya D, Salian SR, Rao SB, Kalthur G, Kumar Pet al. The extent of paternal sperm DNA damage influences early post-natal survival of first generation mouse offspring. Eur J Obstet-GynecolReprod Biol. 2013;166(2):164-7.

10) Wykes SM, Krawetz SA. The structural organization of sperm chromatin. J Biol Chem.2003; 278(32):29471-7.

11) Oliva R.Protamines and male infertility. Hum Reprod Update. 2002; 12(4):417-35.

12) Pittoggi C, Renzi L, Zaccagnini G,Cimini D, Degrassi F, Giordano R.et al. A fraction of mouse sperm chromatin is organized in nucleosomal hypersensitive domains enriched in retroposon DNA. J Cell Sci. 1999;(Pt 20); 112 :3537-48.

13) Zalensky AO, Siino JS, Gineitis AA,Zalenskaya IA, Tomilin NV, Yau P.et al. Human testis/sperm-specific histone H2B (hTSH2B). Molecular cloning and characterization. J. Biol. Chem .2002; 277 (45) :43474-80.

14) Gardiner-Garden M, Ballesteros M, Gordon M, Tam PP. Histone- and protamine-DNA association: conservation of different patterns within the beta-globin domain in human sperm. Mol Cell Biol. 1998; 18(6):3350-6.

15) Bennetts LE, Aitken RJ. A comparative study of oxidative DNA damage in mammalian spermatozoa. Mol. Reprod. Dev. 2005; 71(1):77-87.

16) Villani P, Eleuteri P, Grollino MG, Rescia M, Altavista P, Spanò M.et al. Sperm DNA fragmentation induced by DNAse I and hydrogen peroxide: an in vitro comparative study among different mammalian species. Reproduction. 2010; 140(3):445-52.

17) Varshini J, Srinag BS, Kalthur G,Krishnamurthy H, Kumar P, Rao SB. et al. Poor sperm quality and advancing age are associated with increased sperm DNA damage in infertile men. Andrologia.2012;1:642-9.

18) World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction.2010 5th ed. Cambridge, UK: Cambridge University Press.

19) Singh NP, Stephens RE. X-ray induced DNA double-strand breaks in human sperm. Mutagenesis 1998;13(1):75–9.

20) Vrooman LA, Nagaoka SI, Hassold TJ, Hunt PA. Evidence for paternal age-related alterations in meiotic chromosome dynamics in the mouse. Genetics 2014 ;196(2):385-96.

21) Lee HC, Wei YH. Mitochondrial alterations, cellular response to oxidative stress and defective degradation of proteins in aging. Biogerontology 2001; 2(4):231-44.

22) Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. Fertil Steril.2001;75(2):237-48.

23) Das M, Al-Hathal N, San-Gabriel M, Phillips S, Kadoch IJ, Bissonnette Fet al. High prevalence of isolated sperm DNA damage in infertile men with advanced paternal age.J Assist Reprod Genet.2013;30(6):843-8.

24) Zubkova EV, Robaire B. Effect of glutathione depletion on antioxidant enzymes in the epididymis, seminal vesicles, and liver and on spermatozoa motility in the aging brown Norway rat. Biol Reprod.2004; 71(3):1002-8.

25) Aitken RJ, De Iuliis GN. On the possible origins of DNA damage in human spermatozoa. Mol Hum Reprod. 2010;16(1):3-13.

26) Arnon J, Meirow D, Lewis-Roness H, Ornoy A. Genetic and teratogenic effects of cancer treatments on gametes and embryos. HumReprodUpdate 2001;7(4):394-403.

27) Morris ID. Sperm DNA damage and cancer treatment.Int J Androl.2002;25(5):255-61.

28) Gottschling DE. DNA repair: corrections in the golden years. Current Biology 2006; 16:R956-8.

29) Wyrobek AJ, Eskenazi B, Young S, Arnheim N, Tiemann-Boege I, Jabs EWet al. Advancing age has differential effects on DNA damage, chromatin integrity, gene mutations, and aneuploidies in sperm. Proceedings of the National Academy of Sciences of United States of America 2006;103(25):9601-6.

30) Enciso M, Johnston SD, Gosálvez J. Differential resistance of mammalian sperm chromatin to oxidative stress as assessed by a two-tailed comet assay. ReprodFertil Dev.2011; 23(5):633-7.

31) Haines G, Marples B, Daniel P, Morris I. DNA damage in human and mouse spermatozoa after in vitro-irradiation assessed by the comet assay.AdvExp Med Biol.1998; 444:79-91.

32) Haines GA, Hendry JH, Daniel CP, Morris ID. Germ cell and dose-dependent DNA damage measured by the comet assay in murine spermatozoa after testicular X-irradiation. Biol Reprod. 2002; 67(3):854-61.

33) Demetrius L. Aging in Mouse and Human Systems: A Comparative Study. Annals of the New York Academy of Sciences. 2006; 1067(1):66–82.

34) Demetrius L. Of mice and men. When it comes to studying ageing and the means to slow it down, mice are not just small humans. EMBO Report 2005; 6: S39-44.

35) Zahn JM, Poosala S, Owen AB,Ingram DK, Lustig A, Carter A.et al. AGEMAP: a gene expression database for aging in mice. PLoS Genet. 2007; 3(11):e201.

Review Article

Cryopreservation- A Boon to Assisted Reproductive Technologies

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Chettinad Health City Medical Journal 2018; 7(3): 100 - 105

Abstract

With advancement in assisted reproduction technology, cryopreservation has now become an essential aspect of in vitro fertilization cycle. While this technology was considered to have potential benefit in Assisted Reproductive Technology (ART) earlier, in recent times it has also found its place in fertility preservation with increasing awareness and knowledge of cancer treatment and its consequences. This review gives an overview of importance of cryopreservation technology in infertility treatment and fertility preservation.

Key Words: Cryopreservation; Gametes; Embryo; Gonadal tissue

Introduction

Lazaro Spallanzani, an Italian Physiologist, for the first time in 1776 observed that spermatozoa became motionless when cooled by snow.[1] But a serendipitous discovery by Christopher Polge, paved way for the establishment of sperm cryopreservation by demonstrating the use of glycerol as a cryoprotectant preserved the motility of rooster spermatozoa.[2] The concept of semen banking in humans was first proposed by Mantegazza in 1886, for military mendesiring a progeny,by storing sperm for long periods by cryopreservation.[3] The reporting of the first human pregnancy by frozen sperm (-78°C) by Bunge and Sherman in 1953 was a milestone in infertility leading to the establishment of clinical cryobanks and evolution of cryopreservation protocols in the IVF clinic.[4]

Rapid advancement in ART has led cryopreservation to become an integral part of its treatment, by cryopreserving gametes, embryos and gonadal tissues (Fig 1). This is done by cooling of cells or whole tissues to low, sub-zero temperatures, usually in liquid nitrogen (-196°C) or in liquid nitrogen vapor (-160°C), at which temperature the cells are maintained in a state of suspended animation over a period of time due to the cessation of all metabolic processes within the cell.[5] This is feasible with the use of cryoprotective agents which are employed in cryopreservation to minimize cellular damage during the process of freeze-thawing.

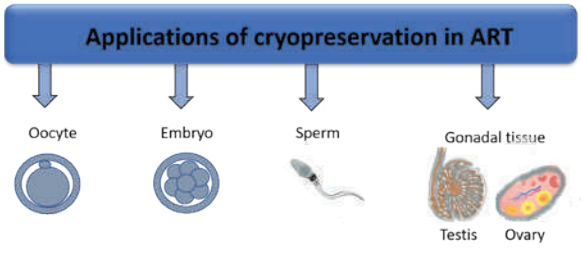

Fig 1: Current applications of cryopreservation in ART

There are two main types of cryoprotective agents insert - penetrating and no-penetrating, based on their ability to permeate through the cell membrane. Commonly used low molecular weight permeating cryoprotectants are glycerol, DMSO, propanediol and ethylene glycol. Non-penetrating agents such as trehalose, raffinose, sucrose, ficoll, plasma proteins and lipoproteins do not permeate the cells but cause dehydration by exosmosis.[6,7]

The different cryopreservation protocols used for gametes/embryos/gonadal tissues are slow freezing, rapid freezing and vitrification. The principle behind all these methods is to first expose the cells to cryoprotectants followed by cooling to the desired storage temperature, and consequently, warming of cells(thawing) along with the removal of cryoprotectants. In slow cooling procedures the cells are equilibrated with penetrating cryoprotectants by exposing them to the increasing concentrations of cryoprotectant solutions and then gradually exposed to temperatures in the decreasing order using a sophisticated automated programmable freezer. This process is usually time consuming.

The rapid cryopreservation method involves plunging of cells into liquid nitrogen after sufficient equilibration with cryoprotectants. This method is relatively simple and does not require any expensive

instruments[8] unlike slow cooling. A more commonly used method in recent times, for oocytes and embryos is vitrification which is an ultra-rapid cooling technique where the cells are exposed to high concentration of cryoprotective agents and then rapidly immersed in liquid nitrogen with a cooling rate as high as 6-10,000° C/min. This protocol is routinely utilized in cryopreserving gametes[2], zygotes[9,10], blastocysts[11,12] and gonadal tissues.[13,14]

To utilize the frozen samples for ART treatment, the cells of interest are thawed when they are taken out of liquid nitrogen and transferred to suitable culture media at 37°C. Rapid rate of thawing is preferred over slow rate as it significantly avoids the growth of intracellular ice crystal formation during this process.

The different biological material that can be cryopreserved in ART treatment and fertility preservation are:

a. Semen

Semen cryopreservation is an established ART procedure which is offered to infertile men undertaking treatment and also men with medical disorders whose treatment might compromise their fertility.Males affected with testicular cancer, Hodgkin's disease and other lymphomas, leukemia, nephrotic syndrome, diabetes, multiple sclerosis, men exposed to pesticides, radiation and mutagenic chemicals and men engaged in military operations are the potential candidates for semen cryopreservation.

Sperm banking is a common application of cryopreservation widely offered in ART, where cryopreserved sperm from anonymous healthy, screened donors can be used in treating infertile couples where the male partner has untreatable azoospermia /or total teratozoospermia. Having a large number of donor samples with different phenotypes and blood groups will help to match the recipient. More importantly, quarantine of samples can be achieved by cryopreservation.

Another application of sperm banking is to store back - up samples for infertile patients undertaking ART treatment. Men are usually required to produce a semen sample on the day of the ART procedure. This can be highly stressful to certain patients and therefore may experience difficulty in collection, due to anxiety-related erectile or ejaculatory dysfunction. This can also be beneficial to patients who might not be present on the day of the procedure due to unscheduled traveling. Storage of sperm as a precautionary measure prior to the scheduled procedure can help to alleviate the emotional stress on such patients and also ensure that treatment can proceed as planned. The common indications for sperm cryopreservation are listed in Table 1.

Indication	Application of cryopreservation
Back-up samples	For patients with difficulty in collection or unavailable on the day of procedure, this option helps to reduce the emotional burden on patients
Donor sperm banking	For heterologous insemination, semen samples can be obtained from donors, as per ICMR guidelines and cryopreserved.
Fertility preservation for cancer patients	Patients with malignant and non-malignant diseases whose treatment can impair fertility can preserve their samples before undergoing treatment. For adult males, semen cryopreservation can be offered.
Social sperm freezing/ convenience banking	For men who would like to start a family later in life can preserve their sperm while they are young
Testicular/Epididymal spermatozoa storage after TESA/TESE/PESA	Patients with obstructive and non-obstructive azoospermia can store retrieved testicular/epididymal spermatozoa samples which can be used for ICSI.

Table 1: Indications for sperm cryopreservation

The process of cryopreservation can lead to dramatic changes in intra-and extracellular environment of the sperm. According to Gao et al., at least 50% of spermatozoa lose their viability due to ultrastructural changes that happens during freeze-thaw process thereby reducing the efficacy of cryopreservation.[15,16] The ultrastructural changes include membrane and acrosome swelling, loss of acrosome and mitochondrial damage.[17-19] The damage to mitochondria and sperm tail during cryopreservation leads to poor motility in post thaw samples[16,20] which is thought to be associated with production of free radicals.[21] The high susceptibility of spermatozoa to reactive oxygen species (ROS) is due to the high PUFA content in their membrane and the limited repair ability.[22-25]

b. Oocyte

Oocyte cryopreservation gives flexibility to ART treatment by allowing the possibility of cryopreserving the oocytes in certain scenarios such as ovarian hyper stimulation syndrome (OHSS) or inability of the male partner to produce viable sperm sample on the day of procedure or for social egg freezing. Oocyte freezing is also offered as a fertility preservation procedure for cancer patients who are at risk of premature ovarian failure due to chemotherapy and/or radiotherapy.[26,27] Another important application of oocyte cryopreservation is in oocyte donation programmes where young fertile women are recruited according to ICMR guidelines[28], undergo controlled ovarian stimulation and oocytes are collected. The obtained oocytes can be cryopreserved and thawed later for indicated patients when required. The indications for oocyte cryopreservation are listed in Table 2.

The human metaphase II oocytes are vulnerable to freeze thaw procedure due to its ultrastructure, large nuclear cytoplasmic ratio, presence of meiotic spindle and sensitivity of zona pellucida.[29] The most common cryoinjuries are damage to the spindle apparatus[30,31],

Indication	Application of cryopreservation
Donor oocyte banking	Oocyte donors can be recruited as per ICMR guidelines. Their oocytes are retrieved and cryopreserved which can be used when needed for indicated after appropriate matching.
Fertility preservation for cancer patients	For women in the reproducible age and prepubertal cancer patients, oocyte cryopreservation can be offered
Social egg freezing	Women who wish to start their family late due to professional, social or financial situations, may choose to freeze their oocytes at a younger age which can later be thawed and used for ICSI.

Table 2: Indications for oocyte cryopreservation

hardening of zona pellucida[32], premature cortical granule reactions[33], chromosomal anomalies[34,35] and parthenogenesis.[36]

To overcome the above stated issues, an alternative strategy is to store immature oocytes at the germinal vesicle stage, obtained from graffian follicles. Immature oocytes are less sensitive to cryoinjury[14] due to their small size (30- 60°m), low metabolic rate, absence of zona, cortical granules and smaller amount of cryosensitive intracytoplasmic lipids.[37] Since these oocytes are at diplotene stage, the lack of spindle apparatus presents less risk of cytogenic errors in subsequent divisions. The only major hurdle for this alternative strategy is that these oocytes have to undergo invitro maturation which may lead to zona hardening.

c. Cryopreservation of gonadal tissues

(i). Testicular tissue

Cryopreservation of spermatozoa retrieved by testicular biopsies is a routinely followed ART procedure for patients with non-obstructive azoospermia which helps to overcome the financial, physical and mental strain of undergoing repeated biopsy procedure. Several studies have shown that the fertilization potential, embryo growth and delivery rate of fresh and frozen spermatozoa retrieved from testicular tissue are comparable.[38-40]

Testicular tissue cryopreservation is also offered for prepubertal cancer patients prior to chemotherapy, as a part of fertility preservation procedure, which is still experimental in nature.[41,42] The prepubertal testicular tissue is highly susceptible to cytotoxic treatments due to the presence of spermatogonia that are proliferative in nature and therefore are targeted by anti-cancer agents.[43] Hence, in these patients, testicular biopsy can be performed and the tissue can be cryopreserved by slow freezing. Fertility restoration can be done by thawing the tissue at a later time, when the patient is ready to have a family, and sperm derived by either auto-transplantation to an orthotopic or heterotopic site or even be matured in vitro.[44]

Indication	Application of cryopreservation
Testicular tissue	Male prepubertal patients can undergo testicular biopsy and cryopreserve their testicular tissue, which can be used in future to restore fertility
Ovarian tissue	Female prepubertal patients can undergo ovarian biopsy and cryopreserve their ovarian cortex, which can be used in future to restore fertility

Table 3: Indications for gonadal tissue cryopreservation

(ii). Ovarian tissue

In women who are at a risk of premature menopause due to several reasons such as recurrent or severe ovarian diseases (cysts, benign tumors, endometriomas), removal of ovary to treat endometriosis or genital cancer[45], chemotherapy or radiotherapy to treat cancer or other systemic diseases[46] and in women with chromosomal anomalies such as Turner's syndrome[45-47], ovarian tissue cryopreservation or whole ovary cryopreservation can be offered. Though this procedure is still experimental in nature, it is a promising alternative to prevent fertility loss in the above mentioned group of patients. It has been shown that ovarian tissue is more cryotolerant and regains function upon transplantation without vascular anastamosis. The indications for gonadal tissue cryopreservation are listed in Table 3.

d. Embryo cryopreservation

Embryo cryopreservation has proved to be a boon to ART as the technique helps in freezing supernumerary embryos, alleviating the stress on patients with OHSS through a frozen embryo transfer cycle, and in patients who are unable to undergo fresh ET due to inadequate endometrial development. A recent addition to the indication for embryo cryopreservation is pre-implantation genetic testing for patients with a history of genetic disease PGD - Preimplantation genetic diagnosis, or in patients with repeated IVF failures who wish to increase the chances of pregnancy PGS- Preimplantation genetic screening. The indications for embryo cryopreservation are listed in table 4.

The outcome of embryo cryopreservation is mainly influenced by the stage of development[48] and the quality of embryo at the time of freezing.[49] Embryos have been successfully cryopreserved at zygote[48], cleavage[50] and blastocyst stages[51], using various freezing protocols with either dimethylsulphoxide (DMSO)[52], 1,2-propanediol(PROH)[53] or glycerol[51] as cryoprotective agents.Currently the most widely used method for cryopreservation of embryos is vitrification using 15%DMSO, 15% EG and 0.5M sucrose.[54] However, the major concern with embryo cryopreservation is the passage of rights and disposal of embryos if the couple divorces each other or in case of death of a partner.

Indication	Applications of cryopreservation
Surplus embryo freezing	Patients who have surplus embryo after IVF/ICSI can have their embryos frozen which can used for their own future use, through an Frozen Embryo Transfer cycle
Patients with deferred ET/ FET cycles	Patients with Ovarian Hyperstimulation Syndrome and whose endometrium is not receptive for an embryo transfer can opt for embryo cryopreservation, which can be thawed and transferred when the patient is ready.
Fertility preservation for cancer patients	If a partner is affected with cancer, the couple can undergo an ART cycle and have their embryos frozen for their own future use.

Table 4: Indications for embryo cryopreservation

Risk of cryopreservation

While cryopreservation is a boon to ART, it also carries with it certain risks such as loss of cell viability, DNA damage, free radical generation and decrease in fertilizing potential of the gametes and reduced implantation potential of the embryos. These exogenous non-physiological processes can change the genetic and epigenetic integrity of the gametes or embryos. Though there are not many reports, the available literature suggests that there is no difference in the postnatal developmental characteristics of progenies born from cryopreserved gametes or embryos.

The same however cannot be said for gonadal tissue as the fertility restoration is still in its infancy. Another major concern is the transmission of infectious agents through liquid nitrogen. It has been shown that infectious agents such as viruses can cross contaminate other stored samples either due to breakage of straws or leakage of samples from sealed ends of plastic straws.[55] The risk of transmission can be significantly reduced by storing the infected specimens in separate liquid nitrogen containers designated for infectious samples.

Conflict of interest: Authors declare no conflict of interest.

References

1) Triana V. Artificial insemination and semen banks in Italy. In: G. David and W Price.editor. Human Artificial Insemination and semen preservation. Newyork, Plenum; 1980. p51,.

2) Polge C, Smith AU, Parkes AS. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature,1949;15;164(4172):666.

3) Bunge RG, Keettel WC, Sherman JK. Clinical use of frozen semen. FertilSteril. 1954; 5(6): 520-9.

4) Bunge RG, Sherman JK. Fertilizing capacity of frozen human spermatozoa. Nature.1953; 24;172(4382):767-8.

5) Mazur P. Freezing of living cells: Mechanisms and implications. AmJPhysiol. 1984;247(3 Pt 1):C125-42.

6) Carroll J, Wood MJ, Whittingham DG. Normal fertilization and development of frozen thawed mouse oocytes: Protective action of certain macromolecules. BiolReprod. 1993;48:606-612.

7) Dumoulin JC, Bergers-Janssen JM, Pieters MH, Enginsu ME, Geraedts JP, Evers JL. The protective effects of polymers in the cryopreservation of human and mouse zonae pellucidae and embryos. Fertil Steril.1994;62(4):793-8.

8) Liebermann J, Nawroth F, Isachenko V, Isachenko E, Rahimi G, Tucker MJ. Potential importance of vitrification in reproductive medicine. BiolReprod.2002;67(6):1671-80.

9) Jelinkova L, Selman HA, Arav A, Strehler E, Reeka N, Sterzik K. Twin pregnancy after vitrification of 2-pronuclei human embryos. Fertil Steril.2002; 77(2):412-4.

10) Selman HA, El-Danasouri I. Pregnancies derived from vitrified human zygotes. Fertil Steril.2002; 77(2):422-3.

11) Yokota Y, Sato S, Yokota M, Ishikawa Y, Makita M, Asada T, et al. Successful pregnancy following blastocyst vitrification. Hum Reprod. 2000; 15(8): 1802-3.

12) Mukaida T, Nakamura S, Tomiyama T, Ishikawa Y, Makita M, Asada T, et al. Successful birth after transfer of vitrified human blastocysts with use of a cryoloop container-less technique. Fertil Steril. 2001;76(3):618-23.

13) Shaw JM, Cox SL, Trounson AO, Jenkin G. Evaluation of the long-term function of cryopreserved ovarian grafts in the mouse, implications for human applications. Mol Cell Endocrinol. 2000; 161(1-2): 103-10.

14) Shaw JM, Oranratnachai A, Trounson AO. Fundamental cryobiology of mammalian oocytes and ovarian tissue. Theriogenology. 2000b; 53(1): 59-72.

15) Gao DY, Mazur P, Critser JK. Fundamental cryobiology of mammalian spermatozoa. In: Karow A,editor. Critser JK, Reproductive Tissue banking. Academic Press, San Diego, CA.1997; 263-328.

16) Watson PF. Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function.Reprod Fertil Dev.1995; 7(4): 871- 891.

17) Wooley DM, Richardson DW. Ultrastructural injury to human spermatozoa after freezing and thawing. JReprod Fert. 1978; 53(2): 389-94.

18) Henry MA, Noiles EE, Gao D, Mazur P, Crister JK. Cryopreservation of human spermatozoa. IV. The effects of cooling rate and warming rate on the maintenance of motility, plasma membrane integrity, and mitochondrial function. Fertil Steril. 1993;60(5):911-8.

19) Medeiros CM, Forell F, Oliveira AT, Rodrigues JL. Current status of sperm cryopreservation: why isn't it better? Theriogenology. 2002;57(1):327-44.

20) O'Connell M, McClure N, Lewis SE. The effects of cryopreservation on sperm morphology, motility and mitochondrial function. Hum Reprod. 2002;17(3):704-9.

21) Duru NK, Morshedi M, Schuffner A, Oehninger S. Cryopreservation-thawing of fractionated spermatozoa and plasma membrane translocation of phosphatidylserine. Fertil Steril. 2001; 75(4): 263-8.

22) Jones R, Mann T, Sherins R. Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides, and protective action of seminal plasma. Fertil Steril. 1979; 31(5): 531-7.

23) Alvarez JG, Touchstone JC, Blasco L, Storey BT. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl.1987; 8(5):338-48.

24) Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J Reprod Fertil.1987; 81(2):459-69.

25) Van Loon AA, Den Boer PJ, Van der Schans GP, Mackenbach P, Grootegoed JA, Baan RA, Lohman PH. Immunohistochemical detection of DNA damage induction and repair at different cellular stages of spermatogenesis of the hamster after in vitro or in vivo exposure to ionizing radiation. Exp Cell Res. 1991; 193: 303-309.

26) Sanders JE, Buckner CD, Amos D, Levy W, Appelbaum FR, Doney K, Storb R, Sullivan KM, Witherspoon RP, Thomas ED. Ovarian function following marrow transplantation for aplastic anaemia or leukaemia. J Clin Oncol. 1988; 6(5): 813-8.

27) Wallace W, Shalet S, Hendry J, Morris-Jones P, Gattamanemi H. Ovarian failure following abdominal irradiation in childhood; radiosensitivity of the human oocyte. Br J Radiol. 1989; 62: 995-998.

28) The Assisted reproductive technologies regulation bill 2010. ICMR. Ministry of health & Family Welfare. Govt. of India. 2010.

29) Carroll J, Depypere H, Matthews CD. Freeze-thaw-induced changes of the zona pellucida explains decreased rates of fertilization in frozen-thawed mouse oocytes. J Reprod Fert.1990;90(2):47- 53.

30) Pickering SJ, Johnson MH. The influence of cooling on the organization of the meiotic spindle of the mouse oocyte. Hum Reprod. 1987; 2(3): 207-216.

31) Aigner S, Van der Elst J, Siebzehnrubl E, Wildt L, Lang N, Van Steirteghem AC. The influence of slow and ultra-rapid freezing on the organization of the meiotic spindle of the mouse oocyte. Hum Reprod. 1992; 7: 857-864.

32) Johnson MH, Pickering SJ, George MA. The influence of cooling on the properties of the zona pellucida of the mouse oocyte. Hum Reprod. 1988; 3, 383- 387.

33) Schalkoff ME, Oskowitz SP, Powers RD. Ultrastructural observations of human and mouse oocytes treated with cryopreservatives. Biol Reprod. 1989; 40: 379-393.

34) Glenister PH, Wood MJ, Kirby C, Whittingham DG. Incidence of chromosome anomalies in first-cleavage mouse embryos obtained from frozen-thawede oocytes fertilized in vitro. Gamete Res. 1987;16(3):205-16.

35) Bouquet M, Selva J, Auroux M. The incidence of chromosomal abnormalities in frozen-thawed mouse oocytes after in-vitro fertilization. Hum Reprod. 1992; 7(1): 76-80.

36) Gook DA, Osborn SM, Johnston WI. Parthenogenetic activation of human oocytes following cryopreservation using 1,2-propanediol. Hum Reprod. 1995; 10(5): 654-8.

37) Oktay K, Karlikaya GG, Aydin BA. Ovarian cryopreservation and transplantation: basic aspects. Mol. Cell Endocrinol. 2000; 169(1-2): 105-8.

38) Ben-Yosef D. Yogev L, Hauser R, Yavetz H, Azem F, Yovel I, et al. Testicular sperm retrieval and cryopreservation prior to initiating ovarian stimulation as the first line approach in patients with non-obstructive azoospermia. Hum Reprod. 1999; 14(7):1794-801.

39) Osmanagaoglu K, Vernaeve V, Kolibianakis E, Tournaye H, Camus M, Van Steirteghem A, Devroey P. Cumulative delivery rates after ICSI treatment cycles with freshly retrieved testicular sperm: a 7-year follow-up study. Hum. Reprod. 2003; 18(9): 1836-40.t

39) Osmanagaoglu K, Vernaeve V, Kolibianakis E, Tournaye H, Camus M, Van Steirteghem A, Devroey P. Cumulative delivery rates after ICSI treatment cycles with freshly retrieved testicular sperm: a 7-year follow-up study. Hum. Reprod. 2003; 18(9): 1836-40.

40) Nicopoullos JDM, Gilling-Smith C, Almeida1 PA, Ramsay JWA. The results of 154 ICSI cycles using surgically retrieved sperm from azoospermic men. Hum. Reprod. 2004; 19(3): 579-85.

41) Anderson RA, Mitchell RT, Kelsey TW, Spears N, Telfer EE, Wallace WH. Cancer treatment and gonadal function: experimental and established strategies for fertility preservation in children and young adults.Lancet Diabetes Endocrinol. 2015 Jul;3(7):556-67.

42) Ethics committee of ASRM. Fertility preservation and reproduction in patients facing gonadotoxic therapies: an Ethics committee opinion. Fertil Steril. 2018;110(3):380-6.

43) Meistrich ML. Male gonadal toxicity. Pediatric Blood Cancer. 2009;53(2):261-6.

44) Guidice MG, Michele de F, Poels J, Vermeulen M, Wyns C. Update on fertility restoration from prepubertal spermatogonial stem cells: How far are we from clinical practice? Stem cell Res. 2017;21:171-7.

45) Wood CE, Shaw JM, Trounson AO. Cryopreservation of ovarian tissue - Potentail 'reproductive insurance' for women at risk of early ovarian failure. Med J Aust. 1997;166(7):369.

46) Oktay K, Buyuk E. The potential of ovarian tissue transplant to preserve fertility. Expert Opin BiolTher. 2002; 2: 361-70.

47) Aubard Y, Piver P, Teissier MP. Indications de la cryopreservation du tissue ovarien. La Presse Medicale 2000; 29: 960-4.

48) Cohen J,De Vane GV, Elsner CW, Fehilly CB, Kort HI, Massey JB, Turner TG. Cryopreservation of zygotes and early cleaved human embryos. Fertil. Steril. 1988; 49: 283-9.

49) Salumets A, Tuuri T, Makinen S, Vilska S, Husu L, Tainio R et al. Effect of developmental stage of embryo at freezing on pregnancy outcome of frozen-thawed embryo transfer. Hum. Reprod. 2003; 18(9):1890-5.

50) Lassalle B, Testart J, Renard JP. Human embryo features that influence the success of cryopreservation with the use of 1,2 propanediol. Fertil Steril. 1985; 44(5): 645-51.

51) Cohen J, Simons RF, Edwards RG, Fehilly CB, Fishel SB. Pregnancies following the frozen storage of expanding human blastocysts. J Vitro Fert. Embryo Transfer 1985; 49(2): 59-64.

52) Mohr LR, Trounson AO. Cryopreservation of human embryos. Ann. N. Y. Acad Sci. 1985; 442(1): 536-43.

53) Quintans CJ, Donaldson MJ, Bertolino MV, Pasqualini RS. Birth resulting from transfer of blastocysts cryopreserved with propanediol after spontaneous hatching. Reprod Biomed Online 2003; 6: 66-8.

54) Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online. 2005; 11(3):300-8.

55) Tedder RS, Zuckerman MA, Goldstone AH, Hawkins AE, Fielding A, Briggs EM, Irwin D, Blair S, Gorman AM, Patterson KG. et al. Hepatitis B transmission from contaminated cryopreservation tank. Lancet 1995; 346(8968): 137-40.

Review Article

Moringa Oleifera- A Wonder Plant for Male Fertility Preservation

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Abstract

Since time immemorial, *Moringa oleifera* Lam. (MO) is a highly valued plant with potential application in the area of nutrition and medicine. All parts of the plant have been used in preparing Ayurvedic medicines while the leaves and pods have been used in preparing traditional dishes. The leaves of this plant are most commonly used as they are rich in amino acids, vitamins, poly-phenols, flavonoids, alkaloids and phytosterols, which attribute to the nutritive and medicinal properties. The tissue and cyto-protective action of MO against various toxic agents is gaining tremendous importance in the field of toxicology. However, very few reports have highlighted the protective effect of MO and its active components against gonadotoxic agents. The present review mainly focuses on the medicinal properties of MO with special reference to its role in male fertility preservation.

Key words: Moringaoleifera, Chemotherapy, Testicular damage, Chemoprotection, Antioxidant

Introduction

Moringa oleifera (MO) is a fast growing tree belonging to monogeneric family Moringaceae, a native of India. The genus Moringa has 13 other species native to the African continent[1] and are distributed in tropical and subtropical regions of the world (Fig 1). The plant has been used from ancient times to prepare various dishes and herbal medicines owing to its rich nutrient contents and therapeutic values. Hence, it is popularly known as 'Miracle tree' or 'Nature gift' or 'Mothers best friend'. In India, MO is commonly referred to as 'drumstick tree', due to the shape of its fruit which resembles a drum stick. It is also called as 'horse radish tree' due to the pungent smell of its roots. The plant has a diverse application such as source of food, animal fodder, natural coagulants, forestry products, fertilizer, alley cropping and fueling.[2]

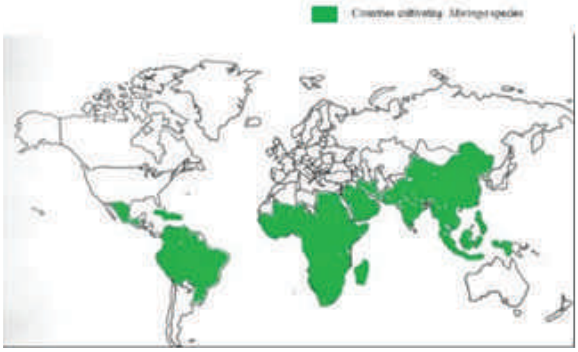

Fig 1: Global distribution of Moringa species (shown in green color)

Taxonomy of *Moringa oleifera* Lam.

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Brassicales
Family: Moringaceae
Genus: *Moringa*
Species: *oleifera*

Moringa oleifera plant as a nutritive source

Moringa oleifera is a highly nutritive plant. MO seeds are rich in monounsaturated fatty acids and proteins with sulfur containing amino acids.[3] Interestingly, Moringa species grow in zones where malnutrition is more evident such as drought affected areas. The leaves, pods and seeds (Fig 2) of this plant are nutritious and most commonly used for preparing traditional dishes. In India, M. oleifera is commercially cultivated mainly for its pods as a vegetable[4]; the pods are rich in proteins, carbohydrates and dietary fibers.[5] The leaves are rich in proteins, essential amino acids, vitamins, minerals and antioxidants which serve as natural remedy in preventing malnutrition in children of under-developed countries.[6-8] A randomized blind placebo control study showed that MO leaf powder can improve nutritional intake and nutritional status in HIV infected patients undergoing anti-retroviral therapy thereby ensuring good immuno-metabolic response.[9]

Fig 2: Different parts of *Moringa oleifera* plant

Use of *Moringa oleifera* in Ayurvedic medicine

Moringa oleifera is the plant of choice for the preparation of various traditional medicines since ancient times. All parts of the plant have shown immense health benefits, particularly in improving function of reproductive, circulatory and immune system. Various studies have shown that MO and its active ingredients, either alone or as a multi-herbal preparation play a promising role in combating a wide range of pathologies including cancer, diabetes, ulcer, oxidative stress, infection and infertility (Table 1). Among different parts of the plant, leaves have been widely studied for its various therapeutic properties as detailed below.

a. Anticancer effect: Aqueous extract of MO leaves hinder the proliferation of alveolar cancer cells by inducing oxidative stress, DNA fragmentation and apoptosis. Moreover, the anti-proliferative effect of the extract was greater in cancer cells than normal cells[10]; thus it can be speculated that an active principle of the extract specifically targets the cancer cells, thereby it can potentially decrease the side effects on normal tissues unlike many of the chemotherapeutic drugs. Aqueous extract of MO leaves increased the cytotoxic effect of chemotherapeutic agents on pancreatic cancer cells[11] by down-regulating nuclear factor-kappa beta (NF-kB) indicating chemo-sensitization of the extract. Ethanolic extract of leaves exhibited anticancer activity in breast and colorectal cancer cell lines.[12] The crude extract and certain isolates of the plant such as glucomoringin isothiocyanate, quercetin and benzyl isothiocyanate have been shown to increase the apoptosis in cancer cell lines.[13-16]

Parts	Composition	Medicinal property
Roots	Benzyl isothiocyanate Phenethyl isothiocyanate	Anticancer Neuro-protective Anti-fertility
Stem (Bark)		Antibacterial action Anticancer effect Insulin sensitization
Leaves	Glucomoringin isothiocyanate, Quercetin, Benzyl isothiocyanate, Vicenin-2, Chlorogenic acid, Gallic acid, Kaempferol, Rosmarinic acid and Rutin	Anticancer Chemo-sensitization
		Anti-diabetic
		Anti-bacterial Anti-viral
		Antianalgesic, anti-inflammatory and antioxidant
		Wound healing
Pods and seeds	Isothiocyanates	Anti-hypertension Antioxidant and antibacterial action Anti-inflammatory action Antiulcer
Flowers	Trypsin inhibitor	Larvicidal action on *Aedes aegypti* (dengue fever)

Table 1: Medicinal properties of different parts of *Moringa oleifera* plant

b. Antidiabetic effects: Methanolic extract of MO leaves prevented diabetes induced nephrotoxicity through its hyperglycemic, antioxidant and anti-inflammatory action.[17] Aqueous extract of leaf showed potent anti-diabetic effect against streptozotocin-induced diabetes in rats.[18] The study on type 2 diabetes-induced mice and rats revealed that the extract of MO exhibited hypoglycemic effect, ameliorated oxidative stress, renal and hepatic dysfunction and improved glycogen synthesis and modulated lipid metabolism.[19-22] It has been demonstrated that MO leaf extract can mitigate alloxan-induced diabetes by regenerating β-cells and modulate the expression of pyruvate carboxylase.[23] Furthermore, the extract from bark showed improvement in insulin resistance induced by dexamethasone.[24]

c. Antimicrobial action: The water-soluble lectin extracted from MO exhibited antibacterial action against *Serrata marcescens* and Bacillus species by inducing cell wall damage.[25] The bark showed significant beneficial effect in the management of urinary tract infections.[26] The aqueous extract from MO leaves showed protection against HBV infection by reducing fibrosis markers, IL-6 and HBsAg secretion.[27] The flower extract inhibited the larval growth of *Aedes aegypti,* a vector of dengue fever, by targeting trypsin and acetyl choline esterase activity.[28]

d Other properties: Aqueous extract of MO leaves contain phytochemicals like vicenin-2, chlorogenic acid, gallic acid, quercetin, kaempferol, rosmarinic acid and rutin which exhibited potential wound healing property.[29,30] The methanolic extract showed analgesic, anti-inflammatory and antioxidant properties in animal models.[31-33] The active compounds in pods and seeds exhibited hypotensive and anti-ulcer properties respectively.[34,35] The hydro-ethanolic extract of MO leaves showed cerebro-protective effect against ischemic stroke by decreasing oxidative stress[36] and penicillin induced convulsion, locomotor behavior by modulating the secretion of neurotransmitters.[37]

Role of *Moringa oleifera* in tissue protection

The tissue protective action of MO is summarized in Table 2. The studies have shown that MO and its active components impart protective action against certain toxic chemicals and radiation. Aqueous extract of MO leaves showed amelioration of radiation-induced oxidative stress in mouse hepatocytes by inhibiting translocation of Nf-kb, decreasing lipid peroxidation and increasing the activity of antioxidant enzymes.[38] The toxic effect of radiation on bone marrow cell was mitigated by the extract by decreasing induction of chromosomal aberrations and micronucleus formation.[39] A recent study revealed that butyl p-hydroxyphenyl-acetate (MIMO2), a novel compound isolated from MO leaves prevented oxidative stress and DNA damage induced by a metal vanadium, a potent neuro-toxicant.[40] Further, MO leaf extract showed nephro-protective effect against acetaminophen, a common analgesic and antipyretic by modulating activity of antioxidant enzymes and anti-inflammatory molecules.[41] Similarly, another study exhibited the mitigating effect of MO leaves on acetaminophen induced hepatotoxicity by restoring glutathione level.[42] The ethanolic extract of MO leaves showed ameliorating effect against anti-tuberculosis drugs (isoniazid, rifampicin, and pyrazinamide) - induced hepatotoxicity.[43] The oil extracted from MO seeds showed protective effect against carbon tetrachloride (CCl_4)-induced hepatitis by decreasing lipid peroxidation and antioxidant status in rat hepatocytes .[44]

MO Extract / Phytochemicals	Toxic agents	Tissue/ cell
Leaf extract	Radiation	Liver
		Bone marrow
Leaf extract	Acetaminophen	Kidney
		Liver
Leaf extract	Antituberculosis drugs- isoniazid, rifampicin, and pyrazinamide	Liver
Leaf extract	Chromium	Testis
Leaf extract	Cyclophosphamide	
Butyl p-hydroxy phenyl-acetate	Vanadium	Neurons

Table 2: Tissue protective action of *Moringa oleifera* leaf extract and its phytochemicals.

Protective role of *Moringa oleifera* in male gonadal toxicity

It is known that certain chemicals and radiation are highly toxic to testes as they affect structure and/or functioning of testes. In literature, there are very few reports on protective action of MO against testicular toxicity. The ethanolic extract of leaves showed the mitigating effect against chromium-induced testicular toxicity and improvement in sperm functions by preventing oxidative stress and increasing testosterone in rats.[45] The oil produced from MO, mitigated mercury induced testicular toxicity by improving steroidogenesis and antioxidant status in rats.[46] However, there are no studies on the protective role of MO against chemotherapy and radiotherapy; which are known to cause severe damaging effects on testes imparting infertility. Therefore, we have conducted experiments to assess the testicular protective effect of ethanolic extract of MO leaves against cyclophosphamide, a broad spectrum anticancer drug.

Role of *Moringa oleifera* in Fertility Preservation

In recent years, the number of cancer survivors have been increasing due to the tremendous advancement in diagnostic and therapeutic strategies in cancer treatment. However, due to the non-specific action of radiation and anticancer drugs, these agents can cause toxicity in normal cells leading to various long-term health issues. Testis is highly vulnerable to these toxic agents due to the presence of rapidly proliferating spermatogonial stem cells (SSC). Depletion of SSC leads to temporary or permanent arrest in spermatogenesis. Therefore, cancer survivors have compromised fertility potential which in turn affects the quality of life post chemotherapy and radiotherapy.

Fertility preservation is a promising option to preserve reproductive capacity of cancer patients undergoing chemo- and radio- therapies. The currently available methods of fertility preservation are depicted in Fig 3.

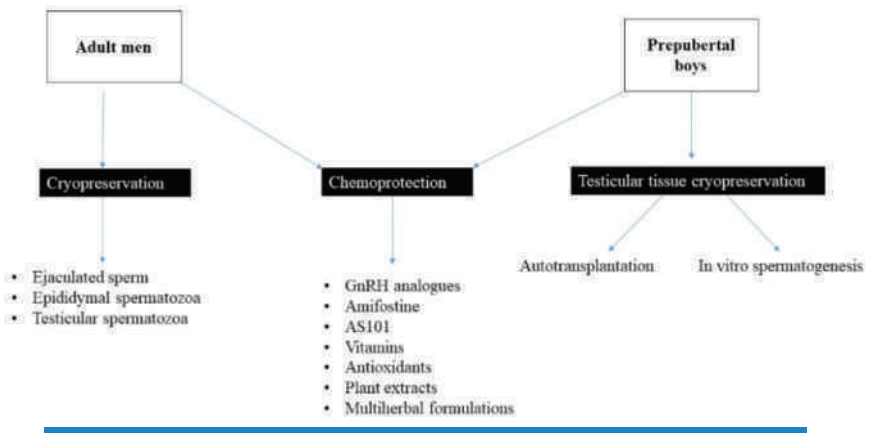

Fig 3: Schematic representation to show fertility preservation options for males

Semen cryopreservation is a well-established method for fertility preservation in adult males. However, it cannot be applicable for pre-pubertal individuals as they do not produce spermatozoa; testicular cryopreservation or SSC cryopreservation followed by derivation of spermatozoa by auto-transplantation or in vitro culture are the only available options. But these protocols are still in the experimental stages and have technical limitations. Since the survival rate in childhood cancer has increased more than 80%[47], there is a need for developing a strategy for fertility preservation, which is practically feasible and economically affordable.

Chemoprotection is considered to be an ideal, alternative option for fertility preservation using cyto-protective agents. These include Gonadotropin releasing hormone (GnRH) analogues[48,49], synthetic compounds like amifostine (WR-2721), AS101 (immuno-modulator)[50,51] and natural products like antioxidants, vitamins and plant extracts.[52-63] Among these, natural products have gained popularity because they are part of our diet and well tolerated by the body. Moreover, the clinical trials with GnRH analogs and other synthetic agents did not show any promising role in preventing chemotherapy-induced male gonadal toxicity.[64,65] The chemoprotective effect of various plant extracts and natural compounds against chemo and radiotherapy induced testicular toxicity are mentioned in Table 3.

Extract/active compounds	Chemotherapeutic agent	Beneficial effect
Zingiber officinale	Busulfan	Increased sperm count and testosterone
Ginkgo biloba	Doxorubicin	Improved sperm functions, decreased oxidative stress and apoptosis
Allium sativum		
Amaranthus viridis	Cyclophosphamide	Improved sperm functions, endocrine functions, antioxidant status and decreased apoptosis
Rosmarinus officinalis	Etoposide	
Podophyllum hexandrum	Radiation	Improved spermatogenesis and antioxidant status
Mentha Piperita		
β-carotene	Methotrexate	Decreased oxidative damage and apoptosis
Curcumin	Cisplatin	
Vitamin C	Cisplatin	
Ellagic acid	Cisplatin Cyclophosphamide	Increased sperm quality, improved spermatogenesis and antioxidant status
Lycopene	Cyclophosphamide	

Table 3: Chemo-protective role of herbal extracts and its active principles in chemo and radiotherapy-induced testicular toxicity

Our earlier study demonstrated that administration of ethanolic extract of MO leaves (MOE) to pre-pubertal mice mitigated the cyclophosphamide (CP) induced testicular toxicity and improved the sperm functional characteristics when mice attained puberty.[66]
The improvements in the testicular functions were associated with decreased lipid peroxidation and increased activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase suggesting that MOE prevents testicular tissue by preventing oxidative stress induced by CP. Further, administration of MOE prior to CP was able to decrease the DNA damage and apoptosis in spermatogonial cells of pre-pubertal mice which are highly sensitive to CP. The protective effect of MOE is further supported by gene expression analysis by quantitative reverse transcriptase PCR (qRT-PCR), where it has been shown to modulate the expression of genes related to DNA damage response, pluripotency and stem cell survival.[67]

Mechanism of protective action

High Performance Thin Layer Chromatography (HPTLC) analysis showed that MOE is rich in quercetin and chlorogenic acid that are known to possess antioxidant and anti-apoptotic properties. CP is an alkylating agent which mainly targets rapidly proliferating spermatogonial cells. In our study, it was demonstrated that MOE increased the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX4), glutathione S transferase (GST) and glutathione reductase (GSR). These enzymes can help in CP-induced oxidative stress and prevent oxidative damage of DNA, RNA and proteins. Single cell gel electrophoresis (Comet assay) and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay showed that DNA damage and apoptosis induced by CP was significantly decreased by MOE administration in spermatogonial cells thereby ensuring the normal spermatogenesis and improvement in quality of spermatozoa. Further, in support of the above findings, flow cytometric analysis showed that MOE attenuated CP-induced depletion of differentiating and haploid population. Gene expression analysis showed that MOE modulated the expression of genes related to apoptosis (P53, Bax, Bcl2 and CytC), pluripotency (Oct4) and stem cell survival (c-Kit) suggesting the molecular mechanism underlying protective effect. The study on kinetics of DNA damage and repair following CP treatment in spermatogonial cells revealed that MOE administration modulated the expression of γ-H2AX (double strand break sensor) as well as RAD51 and KU80 (repair proteins). These observations further confirm that MOE can alter the CP mediated DNA damage response, thereby rescuing the spermatogonial cells leading to normal spermatogenesis (unpublished findings).

Conclusion and future prospects

Administration of MOE seems to be a promising strategy to prevent chemotherapy-induced testicular toxicity. This can serve as an ideal option for pre-pubertal boys undergoing chemotherapy. The use of MO during cancer treatment is expected to have complementary effects due to the anticancer properties of the extract in addition to its normal tissue protective effect. The anti-proliferative activity of the extract helps in reducing the dose of anticancer drugs and in turn can further reduce the testicular toxicity. However, further studies are required to establish the dual role of MO as an anticancer and protective agent.

References

1) Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. Cultivation, Genetic, Ethnopharmacology, Phytochemistry and Pharmacology of *Moringa oleifera* Leaves: An Overview. Int J Mol Sci. 2015;16(6):12791-835.

2) Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: a food plant with multiple medicinal uses. Phytother Res. 2007;21(1):17-25.

3) Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. *Moringa oleifera* Seeds and Oil: Characteristics and Uses for Human Health. Int J Mol Sci. 2016;17(12). pii: E2141.

4) Saini RK, Sivanesan I, Keum YS. Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. 3 Biotech. 2016;6(2):203.

5) Promkum C, Kupradinun P, Tuntipopipat S, Butryee C. Asian Pac J Cancer Prev. 2010;11(3): 627-32.

6) Barichella M, Pezzoli G, Faierman SA, Raspini B, Rimoldi M, Cassani E et al. Nutritional characterisation of Zambian *Moringa oleifera*: acceptability and safety of short-term daily supplementation in a group of malnourished girls. International journal of food sciences and nutrition. 2018: 1-9.

7) Ramaroson RV, Valentin D, Arvisenet G. How to use local resources to fight malnutrition in Madagascar? A study combining a survey and a consumer test. Appetite. 2015; 95: 533-43.

8) Glover-Amengor M, Aryeetey R, Afari E, Nyarko A. Micronutrient composition and acceptability of *Moringa oleifera* leaf-fortified dishes by children in Ada-East district, Ghana. Food Sci Nutr. 2016;5(2):317-23.

9) Tshingani K, Donnen P, Mukumbi H, Duez P, Dramaix-Wilmet M. Impact of *Moringa oleifera* lam. Leaf powder supplementation versus nutritional counseling on the body mass index and immune response of HIV patients on antiretrl therapy: a single-blind randomized control trial. BMC Complement Altern Med. 2017;17(1):420.

10) Jung IL. Soluble extract from *Moringa oleifera* leaves with a new anticancer activity. PLoS One. 2014;9(4):e95492.

11) Berkovich L, Earon G, Ron I, Rimmon A, Vexler A, Lev-Ari S. *Moringa Oleifera* aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. BMC Complement Altern Med. 2013;13:212.

12) Al-Asmari AK, Albalawi SM, Athar MT, Khan AQ, Al-Shahrani H, Islam M. *Moringa oleifera* as an Anti-Cancer Agent against Breast and Colorectal Cancer Cell Lines.PLoS One. 2015;10(8): e0135814.

13) Rajan TS, De Nicola GR, Iori R, Rollin P, Bramanti P, Mazzon E. Anticancer activity of glucomoringin isothiocyanate in human malignant astrocytoma cells. Fitoterapia. 2016;110:1-7.

14) Maiyo FC, Moodley R, Singh M. Cytotoxicity, Antioxidant and Apoptosis Studies of Quercetin-3-O Glucoside and 4-(beta-D-Glucopyranosyl-1-->4-alpha-L-Rhamnopyranosyloxy)-Benzyl l Isothiocyanat e fro m *Moring a oleifer*a. Anticance r Agents Med Chem. 2016;16(5):648-56.

15) Jung IL, Lee JH, Kang SC. A potential oral anticancer drug candidate, Moringa oleifera leaf extract, induces the apoptosis of human hepatocellular carcinoma cells. Oncol Lett. 2015;10(3):1597-1604.

16) Bose CK. Possible role of *Moringa oleifera* Lam. root in epithelial ovarian cancer. MedGenMed : Medscape general medicine. 2007;9(1):26.

17) Omodanisi EI, Aboua YG, Oguntibeju OO. Assessment of the Anti-Hyperglycaemic, Anti-Inflammatory and Antioxidant Activities of the Methanol Extract of *Moringa Oleifera* in Diabetes-Induced Nephrotoxic Male Wistar Rats. Molecules. 2017; 22(4).pii.E439.

18) Yassa HD, Tohamy AF. Extract of *Moringa oleifera* leaves ameliorates streptozotocin-induced Diabetes mellitus in adult rats. Acta Histochem. 2014;116(5):844-54..

19) Tang Y, Choi EJ, Han WC, Oh M, Kim J, Hwang JY et al. *Moringa oleifera* from Cambodia Ameliorates Oxidative Stress, Hyperglycemia, and Kidney Dysfunction in Type 2 Diabetic Mice. Journal of medicinal food. J Med Food. 2017;20(5):502-10.

20) Abd Eldaim MA, Shaban Abd Elrasoul A, Abd Elaziz SA. An aqueous extract from *Moringa oleifera* leaves ameliorates hepatotoxicity in alloxan-induced diabetic rats. Biochem Cell Biol. 2017;95(4):524-30.

21) Paula PC, Sousa DO, Oliveira JT, Carvalho AF, Alves BG, Pereira ML et al. A Protein Isolate from *Moringa oleifera* Leaves Has Hypoglycemic and Antioxidant Effects in Alloxan-Induced Diabetic Mice. Molecules. 2017;22.pii: E271.

22) Olayaki LA, Irekpita JE, Yakubu MT, Ojo OO. Methanolic extract of *Moringa oleifera* leaves improves glucose tolerance, glycogen synthesis and lipid metabolism in alloxan-induced diabetic rats. Journal of basic and clinical physiology and pharmacology. J Basic Clin Physiol Pharmacol. 2015;26(6):585-93.

23) Abd El Latif A, El Bialy Bel S, Mahboub HD, Abd Eldaim MA. *Moringa oleifera* leaf extract ameliorates alloxan-induced diabetes in rats by regeneration of beta cells and reduction of pyruvate carboxylase expression. Biochem Cell Biol. 2014;92(5):413-9.

24) Sholapur HN, Patil BM. Effect of *Moringa oleifera* bark extracts on dexamethasone-induced insulin resistance in rats. Drug Res (Stuttg). 2013;63(10): 527-31.

25) Moura MC, Trentin DS, Napoleao TH, Primon-Barros M, Xavier AS, Carneiro NP et al. Multi-effect of the water-soluble *Moringa oleifera* lectin against Serratia marcescens and Bacillus sp: antibacterial, antibiofilm and anti-adhesive properties. Journal of applied microbiology. J Appl Microbiol. 2017;123(4): 861-74.

26) Maurya SK, Singh AK. Clinical Efficacy of *Moringa oleifera* Lam. Stems Bark in Urinary Tract Infections. Int Sch Res Notices. 2014;2014:906843.

27) Feustel S, Ayon-Perez F, Sandoval-Rodriguez A,Rodríguez-Echevarría R, Contreras-Salinas H, Armendáriz-Borunda J et al. Protective Effects of *Moringa oleifera* on HBV Genotypes C and H Transiently Transfected Huh7 Cells. J Immunol Res. 2017;2017:6063850.

28) Pontual EV, Napoleao TH, Dias de Assis CR, de Souza Bezerra R, Xavier HS, Navarro DM et al. Effect of *Moringa oleifera* flower extract on larval trypsin and acetylcholinesterase activities in Aedes aegypti. Arch Insect Biochem Physiol. 2012 ;79(3): 135-52.

29) Chin CY, Jalil J, Ng PY, Ng SF. Development and formulation of *Moringa oleifera* standardised leaf extract film dressing for wound healing application. J Ethnopharmacol. 2018;212:188-99.

30) Rathi BS, Bodhankar SL, Baheti AM. Evaluation of aqueous leaves extract *of Moringa ole*ifera Linn for wound healing in albino rats. Indian J Exp Biol. 2006;44(11):898-901.

31) Adedapo AA, Falayi OO, Oyagbemi AA. Evaluation of the analgesic, anti-inflammatory, anti-oxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed *Moringa oleifera* in some laboratory animals. J Basic Clin Physiol Pharmacol. 2015;26(5):491-9.

32) Gull I, Javed A, Aslam MS, Mushtaq R, Athar MA. Use of *Moringa oleifera* Flower Pod Extract as Natural Preservative and Development of SCAR Marker for Its DNA Based Identification. Biomed Res Int. 2016;2016:7584318.

33) Jaja-Chimedza A, Graf BL, Simmler C, Kim Y, Kuhn P, Pauli GF et al. Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (*Moringa oleifera*) seed extract. PLoS One. 2017;12(8): e0182658 658.

34) Faizi S, Siddiqui BS, Saleem R, Aftab K, Shaheen F, Gilani AH. Hypotensive constituents from the pods of *Moringa oleifera*. Planta Med. 1998; 64(3):225-8.

35) Kim Y, Wu AG, Jaja-Chimedza A, Graf BL, Waterman C, Verzi MP et al. Isothiocyanate-enriched moringa seed extract alleviates ulcerative colitis symptoms in mice. PLoS One. 2017;12(9): e0184709.

36) Kirisattayakul W, Wattanathorn J, Tong-Un T, Muchimapura S, Wannanon P, Jittiwat J. Cerebroprotective effect of *Moringa oleifera* against focal ischemic stroke induced by middle cerebral artery occlusion. Oxid Med Cell Longev. 2013;2013: 951415.

37) Ray K, Hazra R, Guha D. Central inhibitory effect of *Moringa oleifera* root extract: possible role of neurotransmitters. Indian J Exp Biol. 2003;41(11): 1279-84.

38) Sinha M, Das DK, Bhattacharjee S, Majumdar S, Dey S. Leaf extract of *Moringa oleifera* prevents ionizing radiation-induced oxidative stress in mice. J Med Food. 2011;14(10):1167-72.

39) Rao AV, Devi PU, Kamath R. In vivo radioprotective effect of *Moringa oleifera* leaves. Indian J Exp Biol. 2001 Sep;39(9):858-63.

40) Igado OO, Glaser J, Ramos-Tirado M, Bankoğlu EE, Atiba FA, Holzgrabe U et al. Isolation of a novel compound (MIMO2) from the methanolic extract of *Moringa oleifera* leaves: protective effects against vanadium-induced cytotoxity. Drug Chem Toxicol. 2018;41(3):249-58.

41) Karthivashan G, Kura AU, Arulselvan P, Md Isa N, Fakurazi S. The modulatory effect of *Moringa oleifera* leaf extract on endogenous antioxidant systems and inflammatory markers in an acetaminophen-induced nephrotoxic mice model. PeerJ. 2016;4:e2127.

42) Fakurazi S, Hairuszah I, Nanthini U. *Moringa oleifera* Lam prevents acetaminophen induced liver injury through restoration of glutathione level. Food Chem Toxicol. 2008;46(8):2611-5.

43) Pari L, Kumar NA. Hepatoprotective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. Journal of medicinal food. J Med Food. 2002;5(3):171-7.

44) Al-Said MS, Mothana RA, Al-Yahya MA, Al-Blowi AS, Al-Sohaibani M, Ahmed AFet al. Edible oils for liver protection: hepatoprotective potentiality of *Moringa oleifera* seed oil against chemical-induced hepatitis in rats. J Food Sci. 2012;77(7):T124-30.

45) Sadek KM. Chemotherapeutic efficacy of an ethanolic *Moringa oleifera* leaf extract against chromium-induced testicular toxicity in rats. Andrologia. 2014;46(9):1047-54.

46) Abarikwu SO, Benjamin S, Ebah SG, Obilor G, Agbam G. Oral administration of *Moringa oleifera* oil but not coconut oil prevents mercury-induced testicular toxicity in rats. Andrologia. 2017; 49(1).

47) Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin. 2014;64(2):83-103.

48) Meistrich ML, Wilson G, Huhtaniemi I. Hormonal treatment after cytotoxic therapy stimulates recovery of spermatogenesis. Cancer Res. 1999; 59(15):3557-60.

49) Wang G, Shao SH, Weng CC, Wei C, Meistrich ML. Hormonal suppression restores fertility in irradiated mice from both endogenous and donor-derived stem spermatogonia. Toxicol Sci. 2010;117(1):225-37.

50) Vendramini V, Sasso-Cerri E, Miraglia SM. Amifostine reduces the seminiferous epithelium damage in doxorubicin-treated prepubertal rats without improving the fertility status. Reprod Biol Endocrinol. 2010;8:3.

51) Carmely A, Meirow D, Peretz A, Albeck M, Bartoov B, Sredni B. Protective effect of the immunomodulator AS101 against cyclophosphamide-induced testicular damage in mice. Hum Reprod. 2009;24(6):1322-9.

52) Vardi N, Parlakpinar H, Ates B, Cetin A, Otlu A. Antiapoptotic and antioxidant effects of beta-carotene against methotrexate-induced testicular injury. Fertil Steril. 2009;92(6):2028-33.

53) Ahmed EA, Omar HM, elghaffar S, Ragb SM, Nasser AY. The antioxidant activity of vitamin C, DPPD and L-cysteine against Cisplatin-induced testicular oxidative damage in rats. Food Chem Toxicol. 2011;49(5):1115-21.

54) Bordbar H, Esmaeilpour T, Dehghani F, Panjehshahin MR. Stereological study of the effect of ginger's alcoholic extract on the testis in busulfan-induced infertility in rats. Iran J Reprod Med. 2013;11(6):467-72.

55) Yeh YC, Liu TJ, Wang LC, Lee HW, Ting CT, Lee WL et al. A standardized extract of Ginkgo biloba suppresses doxorubicin-induced oxidative stress and p53-mediated mitochondrial apoptosis in rat testes. Br J Pharmacol. 2009;156(1):48-61.

56) Nasr AY. The impact of aged garlic extract on adriamycin-induced testicular changes in adult male Wistar rats. Acta Histochem. 2017 Jul;119(6):648-662.

57) Ayoka OA, Ojo OE, Imafidon EC, Ademoye KA, Oladele AA. Neuro-endocrine effects of aqueous extract of Amaranthus viridis (Linn.) leaf in male Wistar rat model of cyclophosphamide-induced reproductive toxicity. Toxicol Rep. 2016;3:608-19.

58) Tousson E, Bayomy MF, Ahmed AA. Rosemary extract modulates fertility potential, DNA fragmentation, injury, KI67 and P53 alterations induced by etoposide in rat testes. Biomed Pharmacother. 2018;98:769-74.

59) Samanta N, Goel HC. Protection against radiation induced damage to spermatogenesis by Podophyllum hexandrum. J Ethnopharmacol. 2002;81(2): 217-24.

60) Samarth RM, Samarth M. Protection against radiation-induced testicular damage in Swiss albino mice by Mentha piperita (Linn.). Basic Clin Pharmacol Toxicol. 2009;104(4):329-34.

61) Ilbey YO, Ozbek E, Cekmen M, Simsek A, Otunctemur A, Somay A. Protective effect of curcumin in cisplatin-induced oxidative injury in rat testis: mitogen-activated protein kinase and nuclear factor-kappa B signaling pathways. Hum Reprod. 2009;24(7):1717-25.

62) Turk G, Atessahin A, Sonmez M, Ceribasi AO, Yuce A. Improvement of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid. Fertil Steril. 2008;89(5 Suppl): 1474-81.

63) Ceribasi AO, Turk G, Sonmez M, Sakin F, Atessahin A. Toxic effect of cyclophosphamide on sperm morphology, testicular histology and blood oxidant-antioxidant balance, and protective roles of lycopene and ellagic acid. Basic Clin Pharmacol Toxicol. 2010;107(3):730-6.

64) da Cunha MF, Meistrich ML, Nader S. Absence of testicular protection by a gonadotropin-releasing hormone analogue against cyclophosphamide-induced testicular cytotoxicity in the mouse. Cancer Res. 1987;47(4):1093-7.

65) Vendramini V, Robaire B, Miraglia SM. Amifostine-doxorubicin association causes long-term prepubertal spermatogonia DNA damage and early developmental arrest. Hum Reprod. 2012;27(8):2457-66.

66) Nayak G, Vadinkar A, Nair S, Kalthur SG, D'Souza AS, Shetty PK et al. Sperm abnormalities induced by pre-pubertal exposure to cyclophosphamide are effectively mitigated by Moringa oleifera leaf extract. Andrologia. 2016;48(2):125-36.

67) Nayak G, Honguntikar SD, Kalthur SG, D'Souza AS, Mutalik S, Setty MM et al. Ethanolic extract of Moringa oleifera Lam. leaves protect the pre-pubertal spermatogonial cells from cyclophosphamide-induced damage. J Ethnopharmacol. 2016;182:101-9.

Review Article

Ethical and Social Issues in Fertility Preservation

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Abstract

Fertility preservation is the cryopreservation of gametes (spermatozoa, oocytes), embryos, testicular tissue, and ovarian tissue in order to have a choice of reproduction in the future. This is crucial in patients affected with cancer or other severe medical diseases whose treatment hampers the fertility potential of an individual. Fertility preservation however, raises a lot of ethical issues in each group of patients.

The general limitations that apply to cryopreservation are the quality of the biological material cryopreserved, post-thaw survival rate, and the efficacy & safety of experimental protocols (gonadal tissue and whole ovary cryopreservation). Additionally, there are ethical concerns that impact fertility preservation, like obtaining informed consent from pre-pubertal adolescents with cancer, delaying treatment in cancer patients for the sake of fertility and possible reintroduction of malignancy post auto-transplantation of the frozen-thawed reproductive tissue.

Fertility preservation by cryopreservation of gametes and embryos offers hope to many, while gonadal tissue cryopreservation is still in its infancy and therefore long term follow-up studies are required to monitor the safety of the techniques and the health of children born out of such procedures.

Key words: Fertility preservation, cancer patients, ethical issues, social issues

Introduction

It is a long cherished dream for most people to leave their genetic footprint in this world. All species, including human beings, reproduce and continue to live through their off springs. However, the scenario is not ideal in patients with severe medical conditions.

In various medical conditions, the disease itself or its treatment affects the reproductive organs and thereby fertility of the individual. These medical conditions include cancer -'the emperor of all maladies'[1], Klinefelter's syndrome[2], and non-malignant systemic diseases like systemic lupus erythematosis which requires chemotherapy for treatment.[3] Chemotherapy and radiotherapy are proven to cause gonadal damage as germ cells are extremely sensitive to cytotoxic agents and radiation therapy.[3]

More than 200,000 men and women under the age of 45 years are diagnosed with cancer annually.[4] Until a few decades ago, survival was the only aspect that was focused upon in cancer patients undergoing treatment but now survival rates have increased from 50% (1975-77) to 68% (1999-2006), and the 5-year cancer survival rate in women is currently 90% for breast, 991% for melanoma, 71% for cervical, 69% for non-Hodgkin lymphoma, and 55% for leukemia.[5] This raises the hope for fertility. The American Society of Clinical Oncology now recommends all oncologists to counsel patients regarding fertility preservation options.[6]

The different options available in fertility preservation are cryopreservation of oocytes, spermatozoa, embryos or gonadal tissues for future use through Assisted Reproductive Technologies(ART).

While preservation of gametes and embryos is an established ART procedure, which adults of reproducible age can avail, in prepubertal patients the only available option is cryopreservation of gonadal tissue, which is still experimental in nature.[7]

An overview of the different fertility preservation options are[8]:

1. Sperm cryopreservation: Semen samples can be obtained from adult males through masturbation or coitus interruptus. This is an established procedure routinely used in ART and male fertility preservation.
2. Oocytes: This involves controlled ovarian hyperstimulation followed by transvaginal oocyte aspiration and subsequent cryopreservation of the oocytes obtained. These oocytes can later be thawed and subjected to ICSI (Intra cytoplasmic sperm injection).

3. Embryos: This option requires the woman to undergo an IVF/ICSI cycle after which the developed embryos are cryopreserved and stored for future use.

4. Gonadal tissue: This includes testicular tissue or ovarian tissue cryopreservation which is mostly offered to prepubertal patients. The protocols for fertility preservation in gonadal tissue are still in the experimental stages and therefore come under the blanket of research. Ovarian cortex cryopreservation involves slicing of the ovarian cortex, which contains primordial follicles, into tiny bits and freezing them. In future, they can be thawed and autotransplanted to an orthotopic or heterotopic site or even be matured in vitro. In testicular tissue cryopreservation, a testicular biopsy is done and tissue pieces are frozen. ASRM recommends obtaining institutional review board approval before performing these procedures, since they are experimental in nature.[8]

5. Whole ovary[9]: It is similar to ovarian tissue cryopreservation, but the protocols for this strategy are still in their infancy and hence considered experimental, according to ASRM.

With the help of the above techniques, fertility preservation offers reproductive autonomy to those who may not be able to conceive in the future. However, there are many ethical and social concerns associated with it that need to be borne in mind while offering these services.

Ethical & Social concerns

- Counselling[10]: The oncologists play a very crucial role in helping patients to preserve their fertility. While patients face the battle of a life-threatening disease, the option of preserving fertility may not be their priority at the time of cancer treatment. On the other hand, delaying cancer treatment in order to preserve fertility is also not justified. Due to the short time period between cancer diagnosis and treatment, it can be quite stressful for patients to take a decision to preserve their fertility. It is important for the healthcare providers to provide contextual information concerning their fertility consequences, as each patient's situation is unique.Counseling should be given in a comprehensive manner regarding the available options i.e., oocytes, spermatozoa, embryo, ovarian tissue, whole ovary or testicular tissue cryopreservation.

- Welfare of unborn child: Another concern is the health or life expectancy of the mother who bears the child through fertility preservation after cancer treatment - chances of recurrence of cancer can compromise the welfare of the child. There have also been no long term studies monitoring the health and well-being of children born from gametes obtained from gonadal tissues of a cancer patient. Follow-up studies are also required to monitor and develop the existing protocols with concern to their safety and efficacy.[11]

- Efficacy of experimental procedures[11,12]: In prepubertal children, experimental procedures like ovarian and testicular tissue is currently the only available method for preserving fertility. However, the safety and effectiveness and long-term consequences of usage of such tissue also needs to be determined. Due to the limited evidence to prove its efficacy, the debate arises as to whether it is ethically justifiable to offer such an option to prepubertal cancer patients, which could border on giving false hopes to patients. Hence it is important for the patients and the parents to be informed of the experimental nature and the risks involved. Such treatment should be offered only in institutions which have facilities for long term follow up of these patients and the knowhow of in vitro maturation or gonadal auto transplant and not in run-of-the-mill IVF centres.

- Ovarian stimulation and oocyte retrieval before cancer treatment[13]: In oncological patients of reproducible age, oocyte and embryo freezing is offered to preserve their fertility, before undertaking cancer treatment. However, this option carries with it the risk of ovarian hyperstimulation and oocyte retrieval and most importantly delaying of cancer treatment. The reproductive specialist would be required to plan the procedure at the earliest to avoid delays in commencing cancer treatment, and the ovarian stimulation protocols should be chosen carefully so as not to worsen the disease.

- Informed consent[12]: It is essential for the patient to know the available options in fertility preservation, their success rates and the future risks in addition to the ensuing costs. Comprehensive counselling by the oncologist and reproductive specialist will help the patient make an informed decision. While it is relatively easy to obtain consent from adults, the same is not true in the case of pre-pubertal children and minors under the age of 18, who may not understand the need for fertility preservation nor have the knowledge of the implications or benefits of the procedure. In such cases the parent or guardian can help them take the decision and give their assent. The American Academy of Pediatrics has stated that minors should give their assent 'to the extent of their capacity', to use of any treatments.[14]

- Storage duration and disposal of samples:Few of the major concerns for the ART clinics offering fertility preservation are storage space, duration of freezing, cost involved, and the disposal of the biological material. As the procedure involves conserving fertility for future use, the 'future'can vary from a couple of years to even a decade or more.

With the upcoming demand of fertility preservation, the infrastructure to hold all these materials for an indefinite period of time is a pressing issue. Discarding of the gametes or gonadal tissue poses a big dilemma for reproductive units, especially in case of death during the course of treatment or if the patients do not return to claim their gametes. The ownership of the frozen material in such situations has to be established prior to the treatment to avoid any conflicts.[15]

Conclusion

Fertility preservation is the storage of gametes or reproductive tissue for future use. This can be done either for medical reasons or social reasons. While fertility preservation offers reproductive autonomy, it also raises numerous ethical and social concerns; some of them being the health of children born out of such procedures, the need for long-term safety of experimental protocols, obtaining informed consent from minors and disposal of the frozen biological material. It is important for the healthcare provider to adequately counsel the patients about fertility preservation, especially of the pediatric age group, detailing the risks and benefits in the context of each patient's situation. The primary ethical decrees-beneficence and non-maleficence should be borne in mind by healthcare professionals while offering fertility preservation services. While oncologists have added years to the life of the patient, fertility preservation would add life to the years. Therefore, if ICPD has considered reproduction as a fundamental right[16], then might it not be appropriate to also consider its preservation to be one?

Conflict of Interest: The authors declare no conflict of interest

References

1) Siddhartha Mukherjee. The Emperor of All Maladies: A Biography of Cancer. 2011. Harper Collins, UK.

2) Rives N, Milazzo JP, Perdrix A, Castanet M, Joly-Hélas G, Sibert Let al.The feasibility of fertility preservation in adolescents with Klinefelter syndrome. HumReprod. 2013; 28(6): 1468-79.

3) Sonmezer M, Oktay K. Fertility preservation in female patients. Hum Reprod Update. 2004; 10(3): 251-66.

4) Agarwal SK, Chang RJ. Fertility Management for Women with Cancer. T.K. Woodruff and K.A. Snyder (Eds.). Oncofertility. 2007. Springer.

5) Cancer Facts and Figures, American Cancer Society, Atlanta, GA, USA. 2011. American Cancer Society.Inc.

6) Rodriguez S. Fertile Future? Potential Social implications of Oncofertility. Journal of Clinical Oncology.2013;31(6): 665-7.

7) Anderson RA, Mitchell RT, Kelsey TW, Spears N, Telfer EE, Wallace WH. Cancer treatment and gonadal function: experimental and established strategies for fertility preservation in children and young adults.Lancet Diabetes Endocrinol. 2015;3(7):556-67.

8) Oktay K, Harvey BE, Partridge AH, Quinn GP, Reinecke J, Taylor HS et al. Fertility preservation in patients with cancer: ASCO Clinical practice guideline update. J ClinOncol. 2018;36(19):1994-2001.

9) Silber SJ. Ovary cryopreservation and transplantation for fertility preservation. Molecular Human Reproduction 2012; 18(2): 59–67.

10) Tschudin S, Bitzer J. Psychological aspects of fertility preservation in men and women affected by cancer and other life-threatening diseases. Hum Reprod. 2009; 15(5):587-97.

11) McDougall RJ, Gillam L, Delany C, Jayasinghe Y. Ethics of fertility preservation for prepubertal children: should clinicians offer procedures where efficacy is largely unproven? J Med Ethics.2017;44 (1):27-31.

12) Cohen C. Ethical issues regarding fertility preservation in adolescents and children. Paediatr Blood Cancer. 2009;53(2):249-53.

13) Koch J, Ledger W. Ovarian stimulation protocols for oncofertility patients. J Assist Reprod Genet. 2013; 30(3):203-6.

14) Committee on Bioethics, American Academy of Pediatrics. Informed consent, parental permission, and assent in pediatric practice. Pediatr. 1995; 95(2):314–7.

15) Gracia CR, Gracia J JE, Chen S. Ethical dilemmas on oncofertility: An exploration of three clinical scenarios. Cancer Treat Res. 2010;156:195-208.

16) Policy Recommendations for the ICPD Beyond 2014: Sexual and Reproductive Health & Rights for All. High-Level Task Force for ICPD. 2013. New York.

Seminar Article

Assisted Reproductive Technology for Women Seeking Fertility Preservation

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Introduction

Procreation and parenthood has always been central to human culture and society. There are many who experience situations that affect their future pregnancy adversely. These range from voluntary choice to delay conception till a later date or diseases and treatments that affect future fertility. Earlier there were not too many options for fertility preserving other than planning pregnancy early or cryopreserving sperm. With the advent of assisted reproductive technologies (ART) new tools for fertility conservation have emerged; ovarian cortex, oocyte or embryo cryopreservation. Surgical and pharmacotherapeutic advancements have helped in improving the scope of fertility preservation in oncology.

Indications for Fertility Preservation

- Cancer
- Diseases that can cause premature ovarian insufficiency or testicular failure
- Auto immune diseases
- Prior to myeloablative techniques for hematopoietic stem cell therapy
- Hematologic diseases like thalassemia, sickle cell anemia
- Severe Gonadal damage- iatrogenic/traumatic
- Gender reassignment procedures

Available Fertility Preservation (FP) Strategies

- Cryopreservation Technologies
  - Semen /testicular tissue Cryopreservation
  - Embryo Cryopreservation
  - Mature Oocyte Cryopreservation
  - In-vitro maturation
  - Ovarian Tissue Cryo preservation
- Surgical strategies
  - Fertility sparing surgery
  - Uterine transplant
- Medical adjuvants to chemotherapy
  - Use of gonadotropin-releasing hormone agonists

Cryopreservation Technologies

1) Semen/Sperm cryopreservation

Semen is cryopreserved most often prior to chemotherapy or radiotherapy for cancer as these are known to affect the quality and quantity of gametes significantly. This is the established fertility preservation technique for men who can produce a semen sample. Some men cryopreserve sperm as an 'insurance policy' or 'back up' before a fertility treatment cycle as they may not be able to provide a sample on demand or when the quality of the semen parameters have been highly variable earlier.

Spermatozoa obtained for cryopreservation are obtained by masturbations or after stimulation when with ejaculatory problems, or sperm retrieved by various techniques like Percutaneous epididymal sperm aspiration (PESA), Testicular aspiration (TESA), testicular biopsy and sperm extractionetc.

When an ejaculate is obtained; the semen liquefies, the parameters are analyzed,and motile viable sperms are isolated by swim-up or density gradient methods and then cryopreserved. The sperm pellet obtained after processing is mixed with cryoprotectants and are frozen. Two most commonly used methodsare slow freezing and rapid freezing. The slow freezing technique was introduced by Behrman and Sawada[1] which involves cooling sperm over a period of 2–4 hours in two or three steps, manually or automatically

using a semi programmable freezer. Rapid freezing proposed by Sherman[2] involves exposure of sperm containing straws to nitrogen vapors for 8–10 min and immersion into liquid nitrogen at –196°C.

Smaller number of spermatozoa are obtained in sperm retrieval techniques. They must be cryopreserved by novel approaches involving biological or non-biological sperm carriers. ICSI pipettes, mini straws, cryo loop, agarose microspheres, empty zona pellucida are used as a carrier for freezing small number of spermatozoa.[3-7]

Cryopreservation affects the DNA integrity of some of the stored spermatozoa.[8] Never the less,the cryopreserved sperm have been used over decades to achieve pregnancies safely either by insemination or by IVF/ICSI.

Testicular tissue/sperm preservation in pre-pubertal boys

Testicular biopsy and tissue of immature testis are cryopreserved forpre-pubertal boys in whom semen cryopreservation is not possible. Testicular tissue cryopreservation should be recommended in pre-pubertal boys even though fertility restoration strategies by auto-transplantation of cryopreserved testicular tissue have not yet been tested for safe clinical use in humans.[9]

2) Embryo cryo preservation

Embryo cryopreservation for fertility preservation are opted by the couple where the female partner undergoing ovarian depleting or gonadotoxic treatment of oncologic origin. This is one of the first line strategies for post pubertal fertility preservation and the other being metaphase II (mature) oocyte vitrification which has high success rates9. Women who wants to delay their childbearing but does not want to risk of not having adequate ovarian reserve undergo oocyte cryopreservation. Embryo cryopreservation requires the oocytes to be retrievedfrom the woman and spermatozoa to fertilize the mature oocytes. Hence, the woman needs to go through IVF and should have a source for spermatozoa- husband/partner or donor spermatozoa. The woman may need controlled ovarian stimulation which may delay the cancer treatment. Embryo cryopreservation is not suitable for pre-pubertal patients in whom ovaries are still pre-pubertal.[10]

Embryo cryopreservation provides a good success rate depending on the number and quality of embryos stored. A retrospective analysis done by Cardoza et al in 2015[11] noted a 37% pregnancy rate and 30% cumulative live birth rate in cancer patients who underwent Frozen Embryo Transfer (FET) which was comparable to 43% pregnancy rate and 32% cumulative pregnancy rates (p=0.49 and p =0.85 respectively) in women undergoing IVF for tubal infertility. The cancer patients were found to have significantly higher rates of twinning (p=0.035) which may have been because there were no underlying factors causing infertility.

3) Mature Oocyte Cryopreservation

Oocyte preservation is a good option for women to maintain reproductive autonomy. Metaphase II oocyte vitrification is considered the best technique.[9] Here the woman undergoes controlled ovarian (COS) stimulation, trigger and oocyte retrieval and the mature oocytes are cryopreserved. Hence it goes without saying that mature oocyte cryopreservation is preferred only in women in whom COS is safe and is expected to respond well.

The advancements in the freezing and thawing techniques, especially the introduction of vitrification techniques, have considerably increased the pregnancy rates from cryopreserved, warmed and fertilized oocytes. Cobo et al[12] and Rienzi et al[13] noted that the implantation ratesand pregnancy rates of embryos derived from fresh versus vitrified oocytes were comparable.

Both embryo and oocyte cryopreservation cannot be performed on prepubertal girls as they are not suited for ovarian stimulation or for transvaginal oocyte retrieval.

Ovarian stimulation for embryo or mature oocyte cryopreservation

Ovarian stimulation for fertility preservation is advocated only if it is safe for the patient and ovarian response is expected to be good. Gonadotropin-releasing hormone (GnRH) antagonist protocols are referred as they are shorter, cost effective and yield outcomes comparable to the longer GnRH agonist protocol. Dose of gonadotropins can be decided based on age, ovarian reserve and body mass index to get an appropriate response.

When fertility preservation is to be done especially when the patient has cancer, time constraints may pose limitations in waiting till a conventional day 2 start of COS. In such scenarios, luteal phase stimulation and random start protocols may be used so that the wait till menstruation is avoided. In the luteal phase protocol,GnRH antagonist is given for 3–4 days to achieve a quick down-regulation and controlled ovarian stimulation (COS) is started subsequently with or without the onset of a menstrual bleed. The random start protocol was introduced by Cakmak et al[14] where COS is started as soon as the patient is ready irrespective of the menstrual cycle phase she is in at that point of time. The follicles secondary to the lead follicles are followed and when they reach a size of 12 mm GnRH antagonist control is started till oocyte trigger and retrieval is done. Normal follicular growth and development is observed despite the increased progesterone levels seen in the luteal phase or a spontaneous luteinizing hormone surge, which may occur when the initial lead follicle reaches maturity. The random and conventional start cycles were comparable in the number of eggs retrieved, mature oocyte yield, and fertilization rates. The duration of stimulation is needed till oocyte retrieval are longer compared to the conventional start COS.

Anti-estrogens; letrozole and tamoxifen-based COS or addition to routine COS are associated substantiallyto reduced peak estradiol levels, making them a safer and effective protocol in women with estrogen sensitive cancers.[15-16]

4) In vitro maturation (IVM) of immature oocytes

Here immature oocytes are retrieved either invitro after minimal or no stimulation or ex vivo from ovarian tissue (ovarian cortex biopsy or oophorectomy specimen) followed by IVM and fertilization and embryo cryopreservation or mature oocyte freezing. Ex vivo immature oocyte retrieval and IVM and preservation is a good strategy for prepubertal girls requiring fertility preservation. Immature oocyte aspiration and invitro maturation for IVF is most commonly used in PCOS patients toretrieve more oocytes without ovarian stimulation, thereby completely avoiding the risk of ovarian hyperstimulation syndrome. This is a promising technique to preserve fertility in cancer patients who do not want to risk ovarian stimulation and in prepubertal girls. It also offers hope to preserve fertility in prepubertal girls who have had hematological malignancies like leukemia where ovarian tissue cannot be transplanted due to risk of reintroduction of cancer cells.

First live birth from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient was achieved in Singapore and the case report was published in 2014.[17] The first ongoing pregnancy from fertilization of IVM oocyte was reported in by Segersl et al in 2015.[18] They had retrieved immature oocytes from oophorectomy specimen of 34 patients and had a mean immature oocyte yield of 14per patient. The IVM rate was 36%, fertilization rate 64% and at least one good day 3 embryo could be frozen in seven of eight couples who underwent embryo cryopreservation. One of these patients conceived and had an ongoing pregnancy in 2015.

Giovanna Fasano et al[19] assessed theefficiency of oocyte in-vitro maturation (IVM) and vitrification procedures. They studied 130 adults and six prepubertal girls. A higher mean oocyte yield was obtained from the girls compared with adults (11.5 ± 8.0 versus 3.8 ± 4.2, respectively, $P < 0.001$) but degenerated oocytes were significantly higher in girls (35.5% versus 17.1%, respectively, $P < 0.001$). IVM rates were significantly more in the post pubertal compared to pre-pubertalgirls (28,1% versus10.3%, $P = 0.002$).

5) Ovarian tissue cryopreservation

Ovarian tissue cryopreservation (OTC) is procuring and the procedure involves freezing of ovarian cortical tissue. This technique has many advantages over oocyte and embryo cryopreservation. It does not delay the start of cancer therapy and avoids the risk of ovarian stimulation. There is no need for partner or donor sperm.

The disadvantage is the chance of reintroduction of cancer cells when tissues are auto transplanted. Hence auto transplantation is not adviced in women who have been treated for leukemia. The ovarian cortex is dissected, free cut in to small fragments and are either vitrified or frozen by a slow cooling technique. This ovarian cortex may be harvested leaving the ovaries behind or be done exvivo after performing oophorectomy. OTC can be done both in prepubertal children and adults. The tissue is later warmed and transplanted into the pelvis- either onto ovarian remnant or pelvic peritoneum (orthotopic) or subcutaneously in the fore arm or abdominal wall (heterotopic), when the patient is fit and chooses to regain fertility. Spontaneous pregnancies can occur following orthotopic ovarian tissue transplantation while IVF and oocyte retrieval, fertilization and then embryo transfer is required to achieve pregnancy from the heterotopic cortex transplant.

Complete restoration of ovarian activity has been seen in all women whose transplanted ovarian cortex had primordial follicles. The restored ovarian cortical function has been documented to last up to 7 years following transplant, with the women resuming cyclical menstruation. Jadoul P et al in 2017 reports that more than 86 live births from auto transplanted ovarian tissue have already been documented.[20] They analyzed 545 cases and found that ovarian tissue transplant resulted in a 30%pregnancy rate. Donnez et al in 2004 reported the first live birth following laparoscopic orthotopic transplant of cryopreserved ovarian tissue in a Hodgkin's lymphoma survivor who had gone into premature ovarian insufficiency due to her cancer treatment.The first live birth from orthotopic transplantation of cryopreserved ovarian tissue frozen prior to achieving menarche was reported in 2015.[21] The ovarian tissue was cryopreserved at the age of 14 as the girl needed myeloablative treatment followed by hematopoietic stem cell transplantation to cure severe sickle cell anemia. The first ongoing pregnancy following IVF and ET from a heterotopic auto transplant of cryopreserved ovarian tissue was reported by Stern et al[22] in 2013.

Surgical Strategies

Medical and surgical fertility sparing approaches are applied in cancerpatients so that they have a reasonable hope to conceive naturally or with ART

Fertility sparing Surgery (FSS) in cancer patients

Ovarian transposition in women who need radiotherapy

In women who require pelvic or irradiation laparoscopy/laparotomy and ovarian transposition to above pelvic brim by mobilizing the ovaries after cutting the utero ovarian ligaments. When craniospinal irradiation must be given, the ovaries are fixed as laterally as away from the spine as possible. Titanium clips are placed on the borders of the ovaries for radiological identification. These women require IVF and transabdominal oocyte retrieval when they plan to conceive.

Cervical cancer

Radical trachelectomy (RT), and in selected cases conization and simple trachelectomy have been pioneered to treat early-stage cervical carcinoma as the fertility sparing option. While subsequent pregnancies have been reported, after RT. Fertility may be impaired by anatomical and physiological changes, such as adhesions, cervical stenosis and/or loss of cervical function. The surgical procedure can be followed by subfertility and a need for assisted reproduction.[23]

Ovarian tumors

Fertility-sparing surgery by retaining one ovary or resorting to only partial cystectomy with extensive staging may be offered to young women with stage I ovarian epithelial cancer, after counselling about the risk of recurrence and need for complete surgery after family is completed.

Borderline ovarian tumors (BOT) account for 10–15% of all epithelial tumors, and these women are typically youngerthan 40 years of age when diagnosed. Ithough conservative surgery is done, most of these women require ART techniques in view of altered ovarian reserve or post-operative adhesions. In vitro data have suggested that gonadotrophins and/or high-dose estrogens do not induce proliferation in BOT cell cultures. Hence, IVF may be considered for patients who select conservative fertility-sparing management for borderline tumors as there is no evidence for any adverse effects of pregnancy on the course of BOT.[23]

Endometrial carcinoma

In patients who desire fertility preservation with detailed counselling, conservative management with high-dose progestin treatment may be considered to allow a disease-free window in which to attempt pregnancy.

For younger patients, with a shorter duration of infertility and reassuring ovarian reserve without anovulation or severe male factor, spontaneous conception may be attempted for a limited time but may take several months, which can lead to anxiety about the risk of recurrent disease. Thus, assisted reproductive treatments may be performed for attaining early pregnancy. Efficient ART therapies have helped successful pregnancies to be increasingly reported.[23] The data that are derived from these cases do not seem to show worse prognoses as ART probably increases the chances of gestation and cuts the interval to conception.

Uterine Transplant

Uterine transplant is the surgical procedure where a healthy uterus is transplanted when the uterus is absent congenitally/ iatrogenically. Absolute uterine factor infertility (AUFI) affects approximately one in 500 women of childbearing age. Till the advent of uterine transplantation, adoption or surrogacy were the only options for these women. In 2000, the first human uterine transplant was done in Saudi Arabia although the uterus had to be removed within three hours.[24] The second uterine transplant was successfully done in Turkey in 2011, but although the graft survived under immunosuppressive therapy and the patient resumed menstrual cycles, no live birth occurred although IVF was tried multiple times.[25] The first series of livebirths following uterine transplants from close relatives were reported by the Swedish team headed by Matt Brannstrom in 2014.[26]

Medical Strategy-Gonadotropin releasing Hormone (GnRH) Agonist

"Fertoprotective adjuvant therapy" is a term used for administration of adjuvant therapy during or prior to chemotherapy with an agent that can prevent loss of ovarian reserve. So far, the only drug used in clinical practice is the gonadotropin-releasing hormone (GnRH) agonist.[27-28]

Gonadotropin-releasing hormone agonist causes suppression of the gonadotropin levels to prepubertal levels and decreases utero-ovarian perfusion. It is frequently used in conjunction with chemotherapy hoping to minimize the gameto toxic effects. The GnRHagonist should be started at least 10days prior to chemotherapy so that the suppressive effect is well established, and should be continued till the chemo-therapy is over.[10]

The 2011 Cochrane database suggests considering the 'off label'use of GnRH agonists in women of reproductive age receiving chemotherapy.[29]

Future Perspectives

Activation of follicles

A live birth has been achieved by IVF and ET in a woman with POI using a double approach which resulted in rapid folliculogenesis and mature oocyte production following auto transplantation of ovarian cryopreserved tissues (OTC)

- activating in vivo primordial follicles in by interrupting the Hippo signaling pathway mechanically using ovarian fragmentation/laser/ drilling
- Invitro primordial follicular activation of OCT prior to auto transplantation by acting on the PI3K-PTEN-AKT-FOXO3 pathway

In vitro follicle culture

Cryopreserved tissue transplantation carries the risk of re-seeding cancer cells into the patient. This risk can be minimized by using complete In Vitro Gametogenesis IVG and maturation of oocytes as a means of fertility restoration.

In Vitro Gametogenesis (IVG) by in situ culture of primordial follicles from cryopreserved tissue using three dimensional (3D) multi step culture methods have yielded meiotically competent metaphase II non-human primate and human oocytes. However, considerable researches are needed before the proven safety of using these oocytes.

Artificial ovaries

Instead of above (in vitro culture) primordial follicles may be engineered into an 'artificial ovary', consisting of preantral follicles and other ovarian cells assembled in a 3D matrix, or scaffold. Once transplanted to the patient, this 'artificial ovary' is expected to restore ovarian function and fertility.

New fertoprotective agents

Current researches focuses on agents with anti-apoptotic properties (imatinib, sphingosine-1-phosphate, and tamoxifen) and on agents that also prevent follicle activation such as, an immune modulator (AS101) that acts on the PI3K/PTEN/AKT follicle activation pathway and the anti-Mullerian hormone.[9]

In Vitro spermatogenesis

Spermatogonial stem cells (SSC) are cultured in 3D systems that resemble the in vivo situation. This is still in the early experimental stage.

Artificial gametes

The use of primordial germ cells (PGC) and Pluripotent stem cells (PSC) are potential sources of gametes. It is reported that the generation of haploid mouse spermatid-like cells are able to produce viable and fertile offspring.

Barcelona International Society for Fertility Preservation-eshre-asrm 2015 Expert Working Group Recommendations

- Patients suffering from cancer and non-cancer diseases that may affect their fertility, should be counselled regarding the potential fertility loss and should be referred to fertility specialists to discuss options for FP and current results
- Semen cryopreservation is the only established FP technique in men
- Embryo and oocyte cryopreservation are first-line FP methods in post pubertal women. Metaphase II oocyte cryopreservation (vitrification) is the preferred option.
- Cumulative evidence supports future use of orthotopic transplantation of cryopreserved ovarian tissue to regain ovarian function as well the fertility.
- Testicular tissue cryopreservation should be recommended in pre- pubertal boys even though fertility restoration strategies by auto transplantation of cryopreserved testicular tissue have not yet been tested for safe clinical use in humans.
- The international registries on the short- and long-term outcomes of FP should be established.

References

1) Behrman SJ, Sawada Y. Heterologous and homologous inseminations with human semen frozen and stored in a liquid-nitrogen refrigerator. Fertility and Sterility. 1996;17(4):457-66.

2) Keel BA, Webster BW. Handbook of the laboratory diagnosis and treatment of infertility. CRC Press. 1990.

3) Hsieh Y, Tsai HD, Chang CC, Lo HY. Cryopreservation of human spermatozoa within human or mouse empty zona pellucidae. Fertility and Sterility. 2000;73(4):694–98.

4) Gvakharia M, Adamson G. A method of successful cryopreservation of small numbers of human spermatozoa. Fertility and Sterility. 2001;76:101.

5) Herrler A, Eisner S, Bach V, Weissenborn U, Beier HM. Cryopreservation of spermatozoa in alginic acid capsules. Fertility and Sterility. 2006;85(1)208–213.

6) Isachenko E, Isachenko V, Katkov et al. DNA integrity and motility of human spermatozoa after standard slow freezing versus cryoprotectant-free vitrification. Human Reproduction. 2004;19(4)932–9.

7) Isachenko V, Isachenko E, Montag M et al. Clean technique for cryoprotectant-free vitrification of human spermatozoa. RBMO reproductive biomedicine online. 2005;10(3):350-4.

8) Paoli D, Lombardo F, Lenzi A et al. Sperm cryopreservation: effects on chromatin structure. Adv Exp Med Biol. 2014; 791:137–50.

9) Martinez F, Andersen CY, Barri PN et al. Update on fertility preservation from the Barcelona International Society for Fertility Preservation. Fertility and sterility. 2017;108(3):407-15.

10) Mahajan N. Fertility preservation in female cancer patients: An overview. Journal of human reproductive sciences. 2015;8(1):3-13.

11) Cardozo ER, Thomson AP, Karmon AE, Dickinson KA, Wright DL, Sabatini ME. Ovarian stimulation and in-vitro fertilization outcomes of cancer patients undergoing fertility preservation compared to age matched controls: a 17-year experience. Journal of assisted reproduction and genetics. 2015;32(4):587-96.

12) Cobo A, Kuwayama M, Pérez S, Ruiz A, Pellicer A, Remohí J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertility and sterility. 2008;89(6):1657-64.

13) Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Human reproduction. 2009;25(1):66-73.

14) Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: Random-start controlled ovarian stimulation. Fertil Steril. 2013;100:1673–80.

15) Oktay K, Buyuk E, Libertella N, Akar M, Rosenwaks Z. Fertility preservation in breast cancer patients: A prospective controlled comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. J Clin Oncol. 2005; 23:4347–53.

16) Revelli A, Porcu E, Levi Setti PE, Delle Piane L, Merlo DF, Anserini P. Is letrozole needed for controlled ovarian stimulation in patients with estrogen receptor-positive breast cancer? Gynecol Endocrinol. 2013; 29:993–6.

17) Prasath EB, Chan ML, Wong WH, Lim CJ, Tharmalingam MD, Hendricks M et al. First pregnancy and live birth resulting from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient. Human reproduction. 2013;29(2):276-8.

18) Segers I, Mateizel I, Van Moer E, Smitz J, Tournaye H, Verheyen G et al. In vitro maturation (IVM) of oocytes recovered from ovariectomy specimens in the laboratory: a promising "ex vivo" method of oocyte cryopreservation resulting in the first report of an ongoing pregnancy in Europe. Journal of assisted reproduction and genetics. 2015;32(8):1221-31.

19) Fasano G, Dechène J, Antonacci R, Biramane J, Vannin AS, Van Langendonckt A et al. Outcomes of immature oocytes collected from ovarian tissue for cryopreservation in adult and prepubertal patients. Reproductive biomedicine online. 2017;34(6):575-82.

20) Jadoul P, Guilmain A, Squifflet J, Luyckx M, Votino R, Wyns C et al. Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases. Human Reproduction. 2017;32(5):1046-54.

21) Demeestere I, Simon P, Dedeken L, Moffa F, Tsépélidis S, Brachet C et al. Live birth after autograft of ovarian tissue cryopreserved during childhood. Human reproduction. 2015;30(9): 2107-9.

22) Stern CJ, Gook D, Hale LG, Agresta F, Oldham J, Rozen G et al. First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy. Hum Reprod. 2013; 28:2996–9.

23) Zapardiel I, Cruz M, Diestro MD, Requena A, Garcia-Velasco JA. Assisted reproductive techniques after fertility-sparing treatments in gynaecological cancers. Human reproduction update. 2016;22(3):281-305.

24) Fageeh W, Raffa H, Jabbad H, Marzouki A. Transplantation of the human uterus. Int J Gynecol Obstet. 2002;76:245–251.

25) Erman Akar M, Ozkan O, Aydinuraz B, Dirican K, Cincik M, Mendilcioglu et al. Clinical pregnancy after uterus transplantation. Fertil Steril. 2013;100:1358–1363.

26) Brännström M, Johannesson L, Dahm-Kahler P, Enskog A, Molne J, Kvarnström N. First clinical uterus transplantation trial: a six-month report. Fertil Steril. 2014;101:1228–1236.

27) Woodruff TK. Preserving fertility during cancer treatment. Nat Med. 2009;15:1124–5.

28) Meirow D, Dor J, Kaufman B, Shrim A, Rabinovici J, Schiff E et al. Cortical fibrosis and blood-vessels damage in human ovaries exposed to chemotherapy. Potential mechanisms of ovarian injury. Hum Reprod. 2007; 22:1626–33.

29) Chen H, Li J, Cui T, Hu L. Adjuvant gonadotropin-releasing hormone analogues for the prevention of chemotherapy induced premature ovarian failure in premenopausal women. Cochrane Database Syst Rev. 2011;(11): CD008018.

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'I am your skin
Closer than your kin
I hold you tight like a wife
But for me there is no life

I come in many a shade
On me rests a trillion dollar trade
The fair go to beach
The rest get to bleach!

In the heat I am your fan
And also get to tan
When cold takes hold
I keep you warm in my fold

I let in light
And let out sweat
I give you Vitamin D
And some immunity

I give pain and pleasure
In no small measure
The histamine rush
Makes one to blush

The touch of the feather
Makes you not to bother
Press a little deep
And you begin to weep

In youth the hormonal flow
Makes me to glow
With vagaries of age
One looks like a sage

With reversal of age
The present day rage
The skinner is a winner
And a veritable money spinner!

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