Koch oration 2015

Lead and cadmium as male reproductive toxicants: the known and the unknown

Introduction

Thank you Prof Sivapalan for those kind words of introduction. Let me at the outset thank you and the executive council of the PSSL for inviting me to deliver the ACE Koch memorial oration 2015.I am indeed honoured and humbled by this invitation and the medal you have bestowed on me in memory of a great physiologist. I will cherish this moment and treasure this medal.

It is customary for the orator to speak at least briefly on the person who is commemorated at an oration. As Prof AS Dissanaike said delivering the 9th Koch oration in 1995, "as the years go by, even the orators would scarcely have heard of him". I think we are stepping in to that era now, Hence I have taken most of the information from the presentation made by Prof Carlo Fonseka at the ACE Koch birth centenary symposium.

Arthur Cecil Elseley Koch was born on the 20th of Nov 1903, and was the eldest in a family of four boys. Having received the primary and secondary education at Royal College, Colombo he entered the Ceylon Medical School in 1922. Koch had a brilliant academic record in the medical school, having passed all professional examinations with first class honours and bagging six distinctions and 5 medals. He obtained the Licentiate in 1927. Having placed first, with a first class at the final examination he was awarded a scholarship to study in Britain which he could not utilise due to some family constraint. He joined the department of Physiology in 1935 as a demonstrator. In the same year he was awarded a scholarship to study radiology in Britain. However as the Second World War broke out around this time his scholarship got cancelled.

Although it appears that ACE Koch took to Physiology by default his subsequent achievements and contributions refute this assumption. He continued as an academic in the medical school starting as demonstrator to an asst. Lecturer in 1940, lecturer in 1945, and reader in 1951 on his return from Oxford University. At Oxford University, he researched in the department of human physiology under Prof CG Douglas, the great respiratory Physiologist who invented "the Douglas bag". I quote Prof Douglas on the work ACE Koch has carried out" He has a quick and an

accurate mind, and in dealing with a mass of detail, he does not allow the mere course of the investigation to become obscured".

Prof Koch was appointed to the chair in 1952 becoming the first Ceylonese Professor of Physiology in the Faculty of Medicine, then University of Ceylon which is now University of Colombo. His record of "firsts" are many more including being the First Ceylonese Physiologist to be awarded the fellowship of Royal College of Surgeons without examination in 1966

Much has been said about ACE Koch as a much loved teacher of Physiology. I am sure some of you who are in this audience and many more medical professionals dispersed all over the globe benefitted immensely over a period of one third of a century from his commitment. This experience would have been enriched by his delightful humour and the extreme generosity. The note that Ann Koch, the daughter of ACE Koch who is now in Sydney sent me when I informed her that I will be delivering the oration also parallels what has been said by others and some other aspects of Prof Koch's life.

She wrote "with regards to his working life, I remember being told by my medical student friends that dad's office door in the Phys Dept was always opened to those who needed advice. It was a difficult transition from care free high school where we dealt with rats and earthworms etc to working on human bodies and I was made to understand that dad provided the care and support to many students who were ready to quit in the first few months! I remember the time of a law medical match when the furniture in our lounge room was carried out into the garden by a group of medical students and I saw dad just laughed off the prank even though he identified those who did it. They knew he was a good sport.

The most striking thing in our memory is our dad's humility, compassion and care of, not only his family but also, those he chanced to meet who sought his help. He seemed to have a natural gift to relate across all ages, for example, from the little kid across the road to frail and feeble friends and others needing medical advice. He visited sick relatives in hospital of those who requested his help, like the mechanic who serviced his car and several others.

On a softer note Ann goes on to say, dad married out of his community which resulted in some relatives on both sides not attending their wedding. A marriage made in heaven. Being the romantic he was, every year my mum got a bunch of flowers on their wedding anniversary with his handwritten words "From me to you".

Dad's life revolved around his family and Graham and I are indebted to our parents for the family values they taught us by walking the talk.

President of the PSSL, Past Presidents, executive council of PSSL, My dear teachers and distinguished invitees, today we honour the exemplary life of a great physiologist, educationist, a conscientious man with a compassionate heart, a loyal husband and an adorable father. I believe presenting the findings of the research carried out driven by my instinct to alleviate the pain in the hearts of thousands of couples who suffer silently unable to hold that bundle of joy the dream of their life would be a fitting tribute to him.

Ladies and Gentlemen the topic I have chosen is **Lead and cadmium as male reproductive toxicants: the known and the unknown**

Historical perspectives

Documented evidence of chemicals acting as reproductive toxicants dates back to Roman times.(Cunningham M-AAOHN 1986:34 227-9).Lead has been widely used in plumbing. The symbol of lead, Pb is derived from the word Plumbum. Roman aristocrats enjoyed while washing down platters of lead-seasoned food with gallons of lead-adulterated wine. Later these trends spread to Europe and the far east.

The result, according to many modern scholars, was the death by slow poisoning of the greatest empire the world. Symptoms of "plumbism" or lead poisoning were apparent as early as the first century B.C. Lead has been identified as an abortifacient and a cause of male impotence and infertility. Julius Caesar with all his sexual ramblings was unable to produce more than one known offspring. His successor, Augustus Caesar, is known to have displayed not only total sterility but a cold indifference to sex. Lead and Cadmium containing utensils, moulds and consumables have been used widely since then.

Classification

Reproductive toxicants can be defined as those manmade substances or chemicals released in to the environment which finally leads to hypogonadism and/or infertility. These have been categorised as heavy metals, agricultural chemicals and industrial chemicals. Of the heavy metals, Pb and Cd are the most studied and have harmful effects on the male reproduction. For the purpose of this oration I have therefore, selected the heavy metals Pb and Cd.

Before discussing the effects of Pb and Cd on male reproductive function let me take you through the structure and function of the male RS very briefly.

Structure and function of the male RS

Structure- images1, image 2

Functions .....

The process of spermatogenesis occurs in the walls of the seminiferous tubules. As outlined in the slide the diploid spermatogonia divide to form primary spermatocytes and then undergo a meiotic division to become secondary spermatocytes. With the second meiotic division the secondary spermatocytes become spermatid. Spermatids further mature in the folds of the Sertoli cells and this crucial cellular event is characterised by a series of morphological changes which is called spermiation.

During spermiation, condensation of genetic material in the spermatid occurs forming spermatid nucleus, formation of acrosome surrounding the spermatid head, formation and elongation of the spermatid tail coupled with the packaging of the mitochondria to form the mid-piece of spermatids occur.This process takes approximately 74 days and each spermatogonium produces about 512 spermatids.

I would like to now describe the blood testes barrier which is an important structure for spermatogenesis. BTB is not merely a physical barrier between blood and the developing spermatozoa but a functional barrier that is highly specialised and dynamic coordinating and regulating the processes of spermatogenesis and spermiation.

Why is BTB specialised?

It is located near the basement membrane and is formed by very specialized cell junctions at Sertoli-Sertoli and Sertoli-germ cell interface across the entire seminiferous epithelium. It physically divides the seminiferous epithelium in to basal and apical (adluminal) compartments and is one of the tightest blood tissue barriers in the mammalian body. BTB is fully functional in men after puberty around 12–13 years of age. This novel model of the BTB has been described by Wan and others. The Basal ectoplasmic specialization is formed by testis-specific actin with adherens junction (AJ)] TJs, and gap junctions(GJs), together with desmosomes between adjacent Sertoli cells near the basement membrane.

The actin filament bundles lie perpendicular to the Sertoli cell plasma membrane and are found on both sides of the adjacent Sertoli cells. These networks of actin filament bundles surrounding the TJ and GJ confer the strong adhesive strength to the BTB thus forming a physical barrier.

Why is it a functional barrier?
The BTB restricts paracellular and transcellular transport of substances across and confers cell polarity. It also creates a specialized microenvironment in the adluminal compartment. The BTB also confers the immune privilege status by segregating the adluminal compartment from the systemic circulation. Thus antigens arising in developing spermatids, are ‘‘shielded’’ from the host immune system.Even though the BTB is one of the tightest blood-tissue barriers, it is a highly dynamic ultrastructure because the developing spermatocytes connected in ‘‘clones’’ traverse the BTB at different stages of the cell cycle.

Next function of the male reproductive system is the production, secretion and regulation of reproductive hormones. The hypothalamus secretes GnRH which stimulates the anterior pituitary to secrete FSH, LH. FSH is trophic to the sertoli cells and stimulate to secrete inhibin which in turn inhibits the secretion of GnRH. LH is trophic to Leydig cells which secrete testosterone. T in turn inhibits the secretion of GnRH. Sertoli cells also secrete ABP which helps to keep the T concentrations high within the testis. This process of synthesis, secretion and regulation of male reproductive hormones commences during puberty which is called adrenarche and goes on until andropause.

Pb and Cd are known to adversely affect all three reproductive functions in the male namely spermatogenesis, synthesis, secretion and regulation of reproductive hormones and deposition of sperm in the female genital tract. However, for the purpose of this oration I will not be discussing the process of sperm deposition although this too is known to be adversely affected by the two reproductive toxicants Pb and Cd.

The function of the reproductive system is reproduction. Thus the spermatozoa produced in the male needs to be deposited in the female reproductive system and the sperm have a long and a tedious journey from the vagina until it meets the ovum in the fallopian tube where fertilization occurs.

Justification

There is evidence for decreasing quality of semen for the past 50 years (Ten *et al*.,2008).The rapid increase in Environmental exposure to pollutants due to overwhelming industrialization, urbanization and transportation, occupational exposures to toxicants and Lifestyle factors are thought to contribute for this decline in semen quality and rise in male infertility. Sri Lanka is no exception. Despite evidence of other diseases(CKD), endometriosis known to be caused by environmental toxicants, no studies have been conducted to assess the effects of Pb and Cd on semen parameters.

Methodology (Slides 16,17)

We recruited 300 male partners of infertile couples investigated for infertility at the Vindana reproductive health centre. The demographic data and the information on environmental and occupational exposures were obtained by an interviewer administered questionnaire. Seminal fluid analysis was done according to WHO guidelines and the seminal plasma Pb and Cd were assessed by Graphite Furnace atomic absorption spectophotometry. When categorizing into normozoospermics and pathozoospermics WHO Guidelines were used. ie. if one or more of the sperm parameters were abnormal they were categorized as pathozoospermic.

We randomly selected 20 Pb positive and 20 Cd positive and 20 controls to detect the sperm DNA fragmentation by Halosperm method and the association between DNA fragmentation and Pb and Cd in seminal plasma were determined.

Results

The mean age of the men investigated was 34.8 years with no significant difference in the age and BMI of the patho and the normozoospermics. However the duration of infertility was significantly higher in the pathozoospermic group.

Pb was detected in the 38.3% while Cd was detected in 23%. Both Pb and Cd were detected in 6%

The mean Pb and Cd were 15.77 and 1.18 µg/dl respectively. The pathozoospermics had a higher Pb and Cd concentration although the difference was not statistically significant.

A comparison of Pb and Cd in seminal plasma in other studies is shown in this slide. In the study of Xu et al done in Singapore the Pb levels were lower. However the occupationally exposed men were excluded in this study. In the study conducted in India among the general population the Pb levels were similar but the Cd was much higher. The study in Spain is comparable.

All sperm parameters were lower in the lead positive group although the differences were not statistically significant. Similarly normal sperm morphology and the viability were reduced in the Cd positive men when compared to the Cd negatives.

We compared the sperm DNA fragmentation shown as DNA fragmentation index(DFI%) in Pb and Cd positive men with those who did not have detectable levels of Pb and Cd. The DFI was significantly increased in men positive for Pb and Cd. Here I want to highlight that although there was significant sperm DNA fragmentation, the routinely assessed sperm parameters such as count, motility, normal morphology and the viability were within the normal range.

A DFI of 30% or more is considered high when treating male infertility. Therefore when we further analysed the Pb and Cd levels of the men with DNA fragmentation the Pb levels were significantly higher in men with a DFI of 30% or more.

It was also found that when DFI was above 30%, the sperm motility, morphology and viability reduced significantly as shown in the Table.

In summary we found negative correlation between Pb and Cd in seminal plasma and the routinely assessed sperm parameters although not statistically significant.

Significant positive correlation was found between seminal plasma lead and sperm DNA fragmentation. The negative correlation between sperm DNA fragmentation and all sperm parameters were significant.

What is our inference?

In this group of men, known causes of infertility were excluded. In them although the routinely assessed sperm parameters were normal, failure to fertilize could be due to dysfunction of other non assessed sperm functions such as acrosome reaction and sperm penetration of the ovum and/or sperm DNA damage. This could therefore be caused due to Pb, Cd and other reproductive toxicants. The effects of mixed exposure to Pb and Cd was not assessed due to inadequate sample size(n=6). The effects could be additive or protective.

Similar findings have been reported by many others although the populations and the biological end points studied vary. These have been supported by many laboratory based animal studies and it is conclusive that Pb and Cd are detrimental to the human sperm and its function, which is fertilization.

Now let me discuss some of the mechanisms by which these two reproductive toxicants cause damage to sperm and its functions. They act as Endocrine disruptors on H-P-T axis- thus impairing spermatogenesis and steroidogenesis. In addition direct damage on testis also cause defective spermatogenesis and steroidogenesis Impairment of sperm function is caused by the effects on sperm motility, viability, acrosome reaction, chromosomal abnormalities and DNA damage.

Let’s look at the H-P-T axis.

Pb and Cd act as endocrine disruptors and cause defective steroidogenesis in the testis and its regulation. They act as agonists and/or antagonists of hormones. Testosterone and gonadotropin levels have been measured in exposed and non exposed men. Although the reported results vary, it has been reported that the effects of Cd is more due to accumulation in the testis while Pb is more neurotoxic and effects are more on the hypothalamus and pituitary. This selective accumulation of Pb and Cd on the H-P-T axis disrupts the bidirectional regulatory mechanism of hormone secretion (Lafuente et al., 2001) thus affecting spermatogenesis.

Direct damage to all the cells in the testis also impairs spermatogenesis and steroidogenesis thus causing low sperm counts. Exposure to acute high doses of Pb and Cd has direct cytotoxic effects on all cells in the testis including the blood vessels although high dose exposures are rare. More important are the chronic low dose exposures as most men are exposed to environmental Pb and Cd. A novel model of toxicant induced disruption of spermatogenesis has been forwarded by Wan et al., 2013 where molecular mechanisms that disrupt the structure, function and the dynamism of the BTB are elucidated.

As described earlier the acting filaments are arranged perpendicular to the Sertoli cell membrane in the intact BTB. Wan and his team have shown that on exposure ro Cd, these acting filaments get debundled causing defragmentation and subsequent disruption of the BTB. At the apical Ectoplsmic specialization the effects have been shown with a substance similar to Cd, Adjudin. This caused disruption of apical ES leading to loss of cell polarity and release of immature spermatids in to the luminal compartment due to impairment of spermiation, the process I described earlier.

Progressive motility of sperm is essential for fertilization. Fructose is the substrate of energy to sperm. Lead is thought to alter the fructolysis and ATPase activity in sperm reducing the energy needed for motility. Cd in addition to affecting the mitochondrial structure and function is thought to disturb the microtubule sliding of the sperm tail which is essential for progressive motility. Competing with calmodulin the Ca++ binding protein, is another possible mechanism by which Cd impairs sperm motility. In addition generation of ROS affects sperm motility.

Viability is a measure of sperm cell membrane integrity which is vital to survive in the female reproductive system. Pb and Cd increase production of ROS and decrease the activity and/or quantity of antioxidants. This induces lipid peroxidation and weakens membrane integrity.

Impairment of sperm function is also due to sperm DNA damage. Pb and Cd induce alterations in sperm chromatin structure and decrease chromatin condensation. Condensed nature of sperm chromatin structure protects genetic integrity during the journey through male and female RS By binding to nuclear sulphydryl groups of DNA protamine complexes Pb and Cd delay nuclear decondensation, thus causing fertilization failure. Ladies and gentlemen I have discussed the effects of Pb and Cd on sperm parameters outlining the possible mechanisms.

Now, let’s see how people get exposed to these toxicants. Exposure is mainly though three sources. Environmental exposures occur through contaminated air, water and food. Petroleum products, insecticides, pesticides, pipes, solders, paints, metal utensils, batteries, gun bullets are possible sources. Occupational sources include industries such as painting, printing, plumbing, welding and battery manufacture. Use of Herbal medicines, creams, cosmetics and smoking are some life style sources.

In our study 54.6% of men were exposed to toxicants through environmental or occupational sources. One third of them were exposed to petroleum products and in them the normal morphological forms and the sperm viability were significantly reduced than the not exposed.

What is unknown or doubtful?

Most studies done on reproductive toxicants are on laboratory animals. However Species specific differences are present in the organization and efficiency of spermatogenesis. In humans, spermatogenesis is considered asynchronous and inefficient compared to laboratory animals. Thus, makes the human sperm more vulnerable to effects of toxicants. This needs to be considered in the risk assessment. We also need to be aware that the exposures are mixed. Additive and protective effects of different metals such as Zn and Se have been studied however **some additive and antagonistic effects are not known.**

The effects of reproductive toxicants differ on the stages of life when exposed. Exposures during faetal life, ie in mother are thought to be irreversible while the direct exposures are known to cause reversible effects. The Sertoli cell in the testis is the first cell to develop during sex differentiation followed by proliferation and differentiation of germ cells. There is a surge of Testosterone in the immediate postnatal period followed by a quiescent period until puberty when the T levels increases markedly and reaches the adult levels.

Many factors still remain unknown with regards to effects of exposure during crucial stages of life. Although the number of Sertoli cells and the germ cells are thought to be reduced due to maternal exposure the effects of these are not well known. Testicular germ cell cancer, testicular dysgenesis that are seen in increasing numbers now are thought to be due to maternal exposures. It is postulated that these effects could be seen even up to four generations.

Effects of exposures during the immediate postnatal period and the pre pubertal period where the testis remains quiescent is another area of doubt. The markers of susceptibility also have not been identified.

Image -This model was proposed by Sharpe in his review on Environment and spermatogenesis in 2012. Although it was proposed for persistent organic pollutants whether a similar model applies for Heavy metals is unknown, but possible.

Clinicians reach an impasse when treating the idiopathic infertility group. They have no choice but to resort to IVF/ ICSI. During this process the natural process of selection of the sperm by the ovum is bypassed. The effects on the offspring due to paternal exposure to toxicants are not well studied. These could be reproductive(such as testicular dysgenesis, testiclualr cancer) and non reproductive effects such as autism.

A myriad of proteins/molecules are involved in the signal transduction pathways that cause harmful effects on sperm and its function. There is research going on whether inhibitors of these proteins can be used in the treatment of infertility caused by toxicants.

In spite of exposure to toxicants and high concentrations of heavy metals found in the body fluids, some men are successful in achieving a pregnancy. Are there natural chelators? There is also anecdotal evidence that alternate medicines like ayurvedic preparations enhance sperm functions and the ability to fertilize. The mechanisms of these remain mostly unknown.Large scale studies need to be planned. Collaboration with clinicians, scientists, epidemiologists and complementary medicine physicians may provide more conclusive evidence.

References

Hauser Russ and Socol Rebecca. Science linking environmental contaminant exposures with fertility and reproductive health impacts in the male. Fertility and Sterility: 86,1: 2008

Wan et al., .The apical ES-BTB-BM functional axis is an emerging target for toxicant induced infertility. Trends in Molecular Medicine, July 2013, 19; 7

Ten J, *et. al,* occupational and lifestyle exposures on male infertility: A mini review. The open reproductive Science Journal. 2008; 1: 16 – 21.

Wijesekera GUS, Fernando DMS, Wijeratne S, Bandara N. Environmental and Occupational exposures as a cause of male infertility. Ceylon Medical Journal June: 2015

Wijesekera GUS, Fernando DMS, Wijeratne S, Bandara N. Environmental and Occupational exposures to toxicants in men investigated for infertility. Sri Lanka Journal of Obstetrics and Gynaecology, July: 2014

Evenson DP, Jost LK, Zinaman MJ, Clegg E, Purvis K, de Angelis P, *et al*. Utility of the sperm chromatin structure assay (SCSA) as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 1999;14(4):1039–49.

Yilmaz S, Zergeroglu AD, Yilmaz E, Sofuoglu K, Delikara N and Kutlu P. Effects of sperm DNA fragmentation on sperm parameters and ICSI outcome determined by an improved SCD test, Halosperm. International Journal of fertility and sterility. 2010: 4(2)73-78.

Taha et, al., Correlation between seminal lead and cadmium and seminal parameters in idiopathic oligoasthenozoospermic males. Central European J of Urology

Sharpe RM. Environmental and life style effects on spermatogenesis. Pil Trans. R Society of Biological Sciences, 2012,:365 1697-1712

Acknowledgements

The study was funded by University of Sri Jayewardenepura (ASP/06/P/SCI/2011/12) University Grants Commission (UGC/ICD/RG 2011/05)

Ladies and gentlemen, today I stand before this distinguished audience due to the contribution of several people. I ought to acknowledge at least a few of them.

I thank my research team for their valuable contributions

Dr GUS Wijesekara of the department of health sciences USJP, for his untiring efforts in data collection and data analysis, Prof Sumedha Wijeratne of the Department of Obstetrics and Gynaecology UCFM for her inputs as an expert in the field and Prof Nilanthi Bandara of the Department of Forestry at the Faculty of Applied Sciences, USJP for obtaining the University grant which enabled us to perform the laboratory tests.

The corporation of the study participants is greatly acknowledged and I thank the staff of Vindana reproductive health centre for the technical assistance and support.

The current Dean of the Faculty of Medical Sciences Prof Surangi Yasawardene and the previous deans Prof Mohan de Silva, the current UGC Chairman, Prof Jayantha Jayawardene, Prof Narada Warnasooriya also a former VC at USJP, have always supported me. I sincerely thank all of you for the constant encouragement and being present on this occasion

My Colleagues in the department of Physiology, USJP have generously extended their cooperation to me. I consider it a blessing to work with you and appreciate the trust and confidence you place in me.

The journey I have come from a medical student to Professor in Physiology would not have been possible if not for My Teachers. I thank all of them very sincerely. At this moment it is my duty to thank specially two of my teachers in Physiology. Prof Carlo Fonseka and Prof Colvin Goonaratne had been concerned about my progress every step of the way. Your guidance and encouragement was a great strength to me especially at the time I organised the SAAP conference in 2012. Thank you Sir.

My Ph.D supervisors Prof Harshalal Seneviratne and Prof Jennifer Perera you have not only taught me the basics of research but also taught me the ethics of research. Thank you for instilling these ground rules which are so valuable in guiding my research students.

It was my father who showed me the way to the medical profession and my mother who took me through the long years in the medical school and through my postgraduates studies. My father was a GP who went beyond his practice to serve the people and his profession. Through his simple life style, commitment towards

family and society he taught me to be honest and modest in my personal and professional life. My mother who was a house wife cared for us unconditionally until the last day of her life. She went into detail in doing so. She taught me the importance of quality over quantity and to be courageous in life. Thank you Thatha and Amma

My siblings have been there for me in good times and not so good times. I thank all of you and the extended families for the love, constant encouragement and concern.

Thank you Indrajith for being the wind beneath my wings. I move forwards because of you and your love. Our two children Lakvin and Chrismarie fill my life. I thank them for the happiness and cheer they bring into our lives which energise me to go that extra mile.

Ladies and gentleman I would like to end this oration with these words written to me by Ann Koch, daughter of Prof ACE Koch,

I QUOTE “Something I'll never forget is Carlo Fonseka standing by my side at my dads coffin, with tears rolling down his cheek, and saying to me “he is next to Jesus Christ". These words coming from a so called atheist but as a Christian I know what he meant. Unquote

Being a Christian myself, I know what it meant. I am sure most of you in this audience would also know.

Thank you

Sharaine Fernando