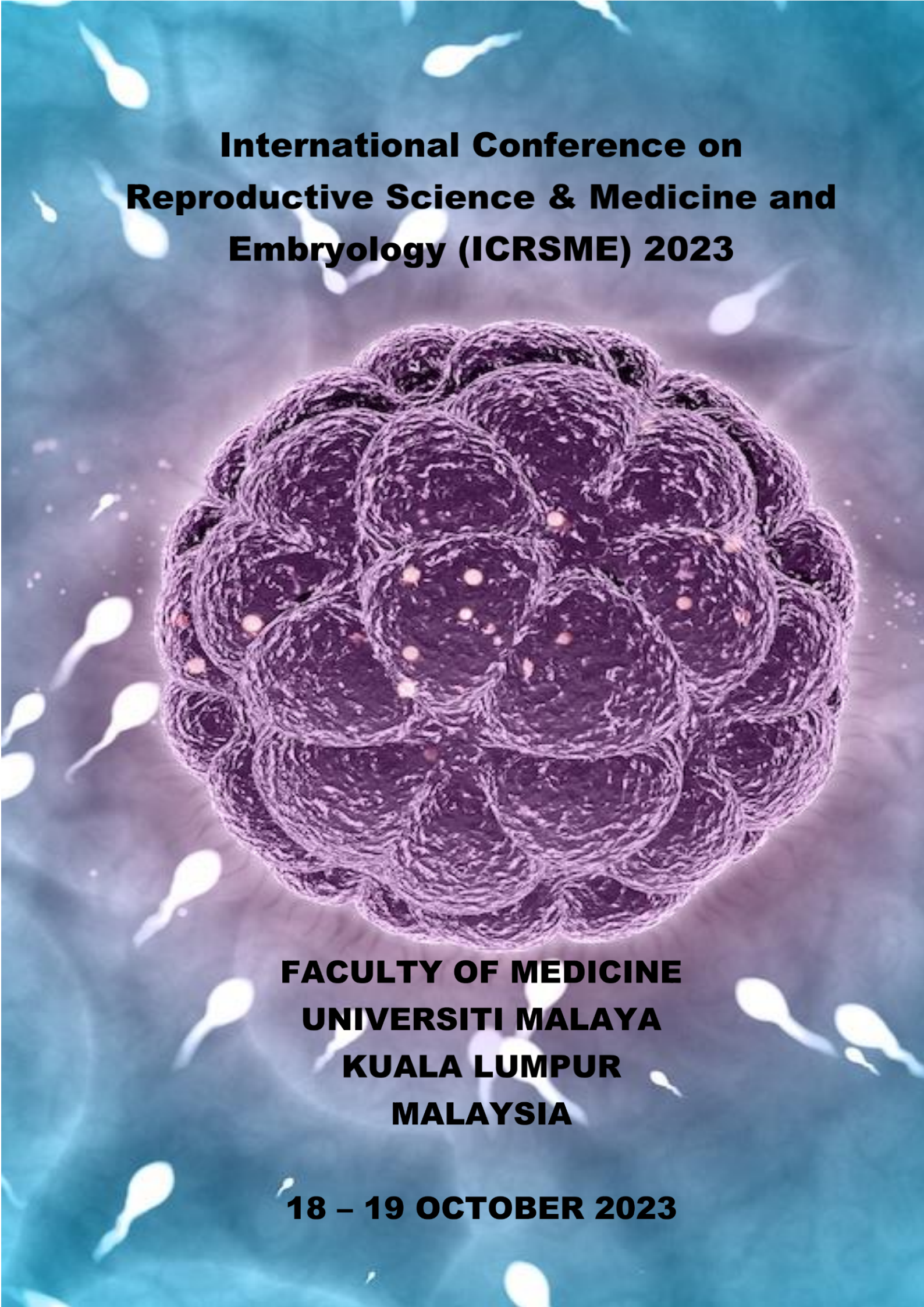
International Conference on Reproductive Science & Medicine and Embryology (ICRSME) 2023



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PROCEEDINGS OF THE INTERNATIONAL CONFERENCE ON REPRODUCTIVE SCIENCE & MEDICINE AND EMBRYOLOGY (ICRSME) 2023

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INTRODUCTION

Reproductive Science is a multidisciplinary field that focuses on understanding the biological processes and mechanisms involved in human and animal reproduction. It encompasses a wide range of scientific disciplines, including reproductive biology, reproductive endocrinology, embryology, and reproductive medicine. Reproductive science plays a pivotal role in advancing our knowledge of reproductive health, fertility, pregnancy, and the prevention and treatment of reproductive disorders. Key areas of interest within reproductive science include (i) Human and animal reproduction which explores the physiological processes of reproduction in humans and animals, including the reproductive cycle, sperm production, fertilization, implantation, pregnancy etc. (ii) Reproductive endocrinology which is a study of reproductive and other hormones and their role in regulating the reproductive system. Reproductive science also includes developing and improving pharmacological and non-pharmacological methods of contraception, enhancing fertility etc. Embryology is a field that encompasses the development of embryos from fertilization through the early stages of growth and embryonic development. Other areas of reproductive science include reproductive genetics where a good understanding is crucial for the investigation of genetic disorders that affect fertility and can be applied in prenatal genetic testing, and genetic counselling. Development of Reproductive Science and Embryology contribute significantly to the advancement of Reproductive Medicine which involves the study of various reproductive disorders and conditions, including infertility, polycystic ovary syndrome (PCOS), endometriosis, etc. and to develop effective diagnostic and treatment strategies. Areas that support reproductive medicine include Assisted Reproductive Technologies (ART) which involves the study of various medical procedures to achieve pregnancy when natural conception is challenging such as *in vitro* fertilization (IVF), intrauterine insemination (IUI), and also include gamete/embryo cryopreservation. Advancements in reproductive sciences have led to significant breakthrough in the diagnosis and treatment of reproductive disorders. For animals, a better understanding of these areas can help to promote breeding programs and conservation efforts, etc. Overall, reproductive science is a vital and constantly evolving field that plays a crucial role in advancing human and animal reproductive health and well-being.

ABOUT ICRSME

This event is dedicated entirely to the area of Reproduction & Embryology, with a goal to showcase research and stimulate collaborations and to help define new paradigms to accelerate research and exchange new knowledge. The ICRSME Conference is focused on understanding the mechanisms that contribute towards successful embryo implantation and embryo development including events in male and female reproductive organs, sperm and ovum, fertilization, embryo transport and development, implantation, and foetal development. In addition, mechanisms underlying male and female infertility will also be discussed. Other aspects include assisted reproductive technologies and pharmacological and non-pharmacological approaches to improve male and female fertility and their reproductive capability. To explore the latest advancements, research, and breakthroughs in this crucial field, ICRSME is deliberated to bring together leading academic scientists, researchers, and research scholars to exchange and share their experiences and knowledge in all aspects including reproductive science, reproductive medicine, and embryology. This conference also provides a premier interdisciplinary platform for researchers, practitioners, and educators to present and discuss the most recent findings, innovations, trends, and concerns as well as practical challenges encountered and solutions adopted in these three related fields. ICRSME is expected to help to accelerate the research and development in this area of health importance. This conference also provides excellent opportunities for interactions and networking among senior scientists, obstetricians & gynaecologists involved in reproductive medicine, assisted reproductive technologies (ART) specialists, public health personnel and academicians besides presenting their own findings and discuss the recent scientific discoveries. This event aims to become the premier platform for the discussion of novel concepts and presentation of latest research related to reproductive science & medicine and embryology. We envision a continuing, annual meeting, in which the specific focus of the conference changes each year, with a format designed to adapt to the needs of this evolving field.

CONFERENCE THEME

A Journey towards successful implantation

This theme is selected to cover the whole aspect of reproductive process which encompass the event in the male reproductive tract and female reproductive tract and development of the fertilized oocyte till embryo implantation, followed by successful pregnancy at the molecular, cellular, tissue, and organismal levels, including its molecular drivers, therapeutic aspects, and the effects of genetic and environmental factors etc on relevant cellular/system properties and interactions across diverse model systems.

CONFERENCE OBJECTIVES

The objectives of ICRSME revolve around advancing knowledge, promoting collaboration, and improving practices in these fields. Among the objectives are to facilitate the sharing of cutting-edge research, scientific discoveries, and clinical advancements related to reproductive science, reproductive medicine and embryology by presentation of original research findings, case studies, and reviews of current literature. Besides, this event is also expected to provide a platform for education and training, allowing participants to enhance their understanding of the latest techniques, technologies, and best practices in reproductive science, medicine and embryology, including workshops sessions. This conference is deliberated to also foster collaboration among researchers, clinicians, scientists, and professionals from various disciplines within reproductive science and medicine which could lead to innovative solutions and approaches to complex challenges and create opportunities for participants to network and establish connections with other colleagues and experts in these fields which can help to potentiate collaborations, research partnerships, and exchange of ideas and knowledges**.**

ORGANIZING COMMITTEE

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PROGRAM OUTLINE

Day 1 (Wednesday 18 October 2023)

8:00 am – 9:00 am	Registration
9:00 am – 9:30 am	Opening Ceremony
9:30 am – 10:15 am	Speaker 1: Prof Dato Dr Zainul Rashid Mohamed Razi Infertility Consultant, UKM Specialist Centre, Kuala Lumpur, Malaysia Topic: An Update on Male Infertility: Causes & Treatment
10:15 am – 10:30 am	Morning tea break
10:30 am – 11:15 am	Speaker 2: Associate Prof Victor Navarro Physiologist, Harvard Medical School, USA Topic: Gonadotropin Releasing Hormone and Female Reproduction: An Update
11:15 am – 12:00 pm	Speaker 3: Prof Arief Boediono Embryologist, IPB University, Indonesia Topic: Embryology: Production of Mouse Diploid Parthenogenetic Embryo through Intracellular Ca^{2+} Regulation
12:00 pm – 1:00 pm	Oral session 1
1:00 pm – 2:15 pm	Lunch break and Poster Presentation
2:15 pm – 3:30 pm	Oral session 2
3:30 pm – 3:45 pm	Afternoon tea break
3:45 pm – 5:00 pm	Oral session 3

Day 2 (Thursday 19 October 2023)

8:00 am – 8:30 am	Registration
8:30 am – 9:15 am	Speaker 4: Prof Sreenivasula Reddy Reproductive toxicologist, Sri Venketeswara University, India Topic: Lifestyle and Environmental Factors affecting Male Reproductive Health – An Overview
9:15 am – 10:00 am	Speaker 5: Prof Diah Tri Widayati Reproductive Technologist, Universitas Gadjah Mada, Indonesia Topic: Additives in Sperm Cryopreservation
10:00 am – 10:15 am	Morning Tea break
10:15 am – 11:00 am	Speaker 6: Associate Prof Nuguelis Razali Infertility Consultant, Universiti Malaya, Malaysia Topic: Female Infertility treatment: An update
11:00 am – 12:45 pm	Oral session 4
12:45 pm – 1:00 pm	Sponsor talk
1:00 pm – 2:15 pm	Lunch break and Poster Presentation
2:15 pm – 4:30 pm	Oral session 5
4:30 pm – 5:00 pm	Award presentation & Closing Ceremony

MESSAGE FROM ICRSME 2023 CHAIRPERSON

I am truly honoured to welcome you all to the International Conference on Reproductive Science & Medicine and Embryology (ICRSME) 2023. It is my privilege to serve as the chairman of this remarkable event, and I am delighted to stand before such a distinguished gathering of experts, researchers, clinicians, and scholars in these three inter-related fields. Without doubt, these fields play an integral role in human life worldwide. Reproductive science and medicine are at the forefront of technological and medical advances, offering hope and solutions to those facing various challenges in fertility, reproductive health, and beyond.

It is our collective commitment to push the boundaries of knowledge, research, and clinical practice that drives us forward and allows us to make a meaningful impact on the lives of countless people. This conference represents an unparalleled opportunity for us to come together, share our experiences, exchange insights, and collaborate on cutting-edge research. Over the next two days, we have an impressive line-up of keynote lectures, oral and poster presentations as well as panel discussions that will provide a platform for showcasing the latest breakthroughs, innovative techniques, and best practices in reproductive science, medicine and embryology. Our goal here is not only to discuss the present state of our field but also to chart a path forward towards a future where reproductive science, medicine and embryology are even more effective, accessible, and inclusive.

I encourage each of you to actively engage in the sessions, ask questions, and contribute your unique perspectives, as it is through our collective efforts that we can drive transformative change. I want to extend my heartfelt gratitude to our distinguished keynote speakers (Prof Dato Zainul and Associate Prof Nuguelis from Malaysia, Associate Prof Navarro from USA, Prof Arief Boediono and Prof Diah Tri Widayati from Indonesia and Prof Reddy from India) who agreed to be here today to share their expertise. I would also like to thank Lab IVF as our main sponsor, and all the dedicated individuals who have worked tirelessly to make this conference a reality. Your dedication and hard work have been instrumental in bringing us all together, and we owe you a debt of gratitude for your unwavering commitment to the advancement of reproductive science, medicine and embryology.

As we embark on this exciting journey of discovery and collaboration, let us remember the profound impact that our work can have on the lives of individuals and families. In closing, I want to express my sincere hope that this conference will be a source of inspiration, learning, and connection for all of us. Together, we can continue to push the boundaries of what is possible in reproductive science, medicine and embryology, and ultimately, improve the lives of those we serve. Thank you to all participants and for your contributions to our shared mission.

Associate Professor Dr Naguib Bin Salleh

Chairman, ICRSME 2023

Head of Department of Physiology, Faculty of Medicine, Universiti Malaya

MESSAGE FROM THE DEAN OF FACULTY OF MEDICINE, UNIVERSITI MALAYA

It is with great pleasure and enthusiasm that I welcome you to the International Conference on Reproductive Science & Medicine and Embryology (ICRSME) 2023. This gathering represents a significant milestone in our pursuit of knowledge and innovation in these fields, and we are glad to share with you the exciting journey of discovery, collaboration, and progress. The world of reproductive science and medicine is constantly evolving, and our ability to understand, manipulate, and optimize the processes involved in human reproduction has never been more advanced. From breakthroughs in assisted reproductive technologies, to pioneering research in reproductive health and fertility preservation, our community continues to push the boundaries of what is possible.

This conference serves as a unique platform for experts from diverse backgrounds – scientists, clinicians, researchers, educators, and industry leaders – to come together, share their insights, and foster interdisciplinary collaborations. It is through these partnerships that we can collectively tackle the most pressing challenges in reproductive science and translate our findings into meaningful advancements that benefit individuals and society as a whole. Over the next few days, we have a packed agenda that includes keynote lectures, panel discussions, oral presentations, and poster sessions. These presentations will not only showcase the latest discoveries but also provide opportunities for lively discussions and knowledge exchange. I encourage each of you to actively engage in these sessions, ask questions, challenge assumptions, and explore new perspectives.

This conference would not have been possible without the efforts of many. From the distinguished speakers, organizing committee, to volunteers and sponsors, all have worked tirelessly, with dedication and commitment, to shape this event. It is important to appreciate the impact our work in reproductive science and medicine can have on individuals, families, and society as a whole. It is our duty to ensure that the knowledge we generate, and the innovations we develop, are used for the betterment of humanity.

It is in this spirit that I hope this conference will foster exciting discussions and collaborations. Let us embrace this opportunity to ignite new ideas, inspire one another, and contribute to the advancement of reproductive science and medicine. Thank you for your presence and participation. Together, we will continue to push the boundaries of what is possible in these fields.

Professor Dr. April Camilla Roslani

Dean, Faculty of Medicine, Universiti Malaya

KEYNOTE SPEAKERS

Prof. Dato' Dr. Zainul Rashid Bin Mohamad Razi, MOG, FRCOG (Lon), FICS (USA), Medical Doctrate (Nottingham), AM (Malaysia) has more than 35 years of experience in Obstetrics and Gynaecology, and specializes in Reproductive Medicine and Infertility treatment. He is an expert in the areas of human *in-vitro* fertilization & embryo transfer and artificial insemination.

Prof Arief Boediono, DVM, PhD is currently the Head of Laboratory of Embryology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia. He is also the President of Indonesian Society of Human Embryology (ISHE)r. His research interest includes Embryo Biotechnology, Stem Cell and Cryopreservation. He has been involved in the conservation of Malaysia's last living Sumatran rhinoceros in 2019.

Associate Prof Victor M Navarro PhD is an Endocrine & Reproductive Physiologist at Harvard Medical School, USA. His research interest is on the study of the neuronal mechanisms that control reproductive function. His research expands all stages of development, from the events that participate in the differentiation of the brain into masculine or feminine during perinatal periods, to puberty onset and reproductive success in adulthood

Prof Diah Tri Widayati Ir. M.P., Ph.D., IPM is a Professor of Assisted Reproductive Technology in Universitas Gadjah Mada, Indonesia. She specializes in the field of Assisted Reproduction Technology in animals as well as in reproductive immunology. Among her research include comparing the fertility between different goat species after estrous synchronization and artificial insemination.

Associate Prof Nuguelis Razali MBBS (UM), MObGyn (UM) is Infertility Consultant at Universiti Malaya, Malaysia. Her research interest is on topics including comparative characteristics of spermatozoa harvested and cryopreserved in culture and cryoprotectant media with or without donor serum proteins. She has also identified how endometriosis affect oocyte and embryo quality in the perspectives of *in vitro* fertilisation cycles

Prof P. Sreenivasula Reddy PhD is an eminent scholar, a dynamic and dedicated researcher in the field of Reproductive Toxicology at Sri Venkateswara University, Andhra Pradesh, India. He is a known world expert in reproductive toxicology and among his work include investigating the effect of environmental compounds on male and female reproductive system.

MALE INFERTILITY – AN OVERVIEW

Prof. Dato' Dr. Zainul Rashid Bin Mohamad Razi, MOG, FRCOG (Lon), FICS (USA), Medical Doctorate (Nottingham), AM (Malaysia)

Male infertility has been a neglected subject. They are seldom examined, the men seldom come for clinic follow-up and many clinicians are not familiar with the treatment of Male Infertility such that when men with gross infertility present themselves to the clinicians, they are told to go away. Who should be managing this sub-speciality is still debatable up to this day? And yet, the problem of Male Infertility is increasing by the day due to the current lifestyle, diet, environment and habits which are detrimental for normal sperm production and development. The treatment available for male infertility is rather limited giving clinicians few choices to assist them. Current exposure to toxic atmosphere, high temperature, STD infections, recreational drugs, additives in processed food, clothing style and many others have largely contributed to the production of low-quality sperm. The current situation of poor semen quality has forced WHO to produce a new guideline for normal semen of modern men with lower levels of count, movement and shape. Otherwise, more than half of the world's men population will be classified as sub fertile. Male infertility can be due to no production, poor quality of spermatozoa, azoospermia in ejaculate, anejaculation or production of immature sperm. For men who cannot ejaculate due to neural defect, they can be assisted to ejaculate by stimulation of the prostate digitally or by electrical means. Some men produce adequate sperm but are immotile either due to production of entirely dead sperm, live but immotile sperm such in Kartagener's Syndrome or presence of antibody towards his own sperm. A special solution can be introduced to determine whether the sperm are still alive or otherwise. Any life spermatozoa, however few, can be used for fertility treatment. As for men with anti-sperm antibody, the use of low dose steroids can reduce his antibody production thus allowing normal fertilization to occur. For azoospermic men, it could be totally due to no production, blocked epididymis due to congenital Absence of the vas deferens (CAVD) or blocked epididymis due to infection, surgery or injury. A rare condition is retrograde ejaculation into the bladder. Treatment of Male infertility can be via Intrauterine insemination (IUI), In-vitro fertilization or Intracytoplasmic Sperm Injection (ICSI) depending on the semen quality. Fortunately, with current armamentarium available in the treatment of Male Infertility, they can be helped provided the Female partner can produce sufficient oocytes. With ICSI, these sub fertile Males can be assisted as long as they can produce 'live' spermatozoa which can be used for direct injection of a single spermatozoa into each matured oocyte.

GONADOTROPHIN RELEASING HORMONE AND REPRODUCTION – AN UPDATE

Associate Prof Victor M Navarro PhD

Gonadotropin releasing hormone (GnRH) is released from neurons located in the preoptic area in a tonic (pulsatile) manner, leading to pulses of luteinizing hormone (LH) from puberty onset onwards in both sexes. Additionally, in females, there is a surge-like release that is required for ovulation and that is under the control of sex steroids (positive feedback) and circadian cues. Despite the critical role of both modes of GnRH release, pulsatile and surge, for sexual development and reproductive success, little is known about the neuroendocrine mechanisms governing GnRH release. In this context, kisspeptins, secreted from two distinct populations of hypothalamic Kiss1 neurons have become a key regulator of both modes of GnRH release. On one hand, Kiss1 neurons of the arcuate nucleus, which co-express neurokinin B (NKB) and dynorphin A (Dyn), also termed KNDy neurons, control the pulsatile release of GnRH. On the other hand, Kiss1 neurons of the anteroventral periventricular nucleus continuum (AVPV/PeN), exclusive to the female brain, control the pre-ovulatory LH surge. Our lab has significantly contributed to the characterization of these regulatory processes of kisspeptin and GnRH release in health and disease. Understanding these mechanisms is critical in the development of new treatments for disorders derived from central impairments of gonadotropin release, such as precocious/delayed puberty, hypothalamic amenorrhea or polycystic ovarian syndrome (PCOS), and to improve IVF outcomes.

PRODUCTION OF MOUSE DIPLOID PARTHENOGENETIC EMBRYO THROUGH INTRACELLULAR Ca^{2+} REGULATION

Prof Arief Boediono, DVM, PhD

Fertilization between oocytes and sperm *in vivo* and *in vitro* involves complex mechanisms. The mechanism starts with the activation of the oocyte through the release of PLCζ sperm into the oocyte, to catalyze the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2). PIP2 hydrolysis produces two-second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (InsP3). Furthermore, InsP3 binds to its receptor located on the endoplasmic reticulum, resulting in the release of Ca^{2+} in the endoplasmic reticulum and forming an increased intracellular pattern of Ca^{2+} (Ca^{2+} oscillation). Ca^{2+} oscillation in the oocyte resulted in increased activity of calmodulin-dependent protein kinase II gamma (CaMKIIγ). These conditions affect the decrease in the expression of maturation promoting factor (MPF) which causes the meiotic resumption in the oocyte to the anaphase stage. The increase in CaMKIIγ activity also affects the decrease in the expression of mitogen-activated protein kinase (MAPK), which results in the formation of a female pronucleus. Furthermore, the increase in CaMKIIγ activity also affects the formation of polar body II. The importance of the role of Ca^{2+} oscillation in oocyte activation has resulted in researchers performing artificial oocyte activation (AOA) to stimulate Ca^{2+} oscillation. Common chemicals used to stimulate artificial oocyte activation include ethanol, strontium chloride, probol ester, thimerosal, puromycin, ionomycin, and calcium ionophore (A23187). AOA can be applied to the production of parthenogenetic embryos. Parthenogenesis embryos are embryos produced without the process of fertilization. The production of parthenogenesis embryos was performed through AOA combined with post-activation treatment using cytochalasin B, which plays a role in inhibiting the formation of polar body II in oocytes. This condition causes the resulting in diploid parthenogenetic embryo, that can develop to the blastocyst stage and are capable of implantation. However, diploid parthenogenetic embryos are unable to develop post-implantation. This is due to the genome imprinting mechanism that occurs during the process of embryonic development. Based on these conditions, the diploid parthenogenic embryos can be used as an alternative source of embryonic stem cells. Our results showed that embryonic stem cells produced from diploid parthenogenetic mouse embryos were able to differentiate into neuron-like cells.

LIFESTYLE AND ENVIRONMENTAL FACTORS AFFECTING MALE REPRODUCTIVE HEALTH – AN OVERVIEW

Prof P. Sreenivasula Reddy PhD

During the past few decades many reports have suggested that the male reproductive health has steadily deteriorated in both wild life and humans. The contributory factors for this deterioration have been traced to changes in the life style and exposure to environmental, industrial, and occupational chemicals, therapeutics etc. Stressed lifestyle and exposure to environmental pollution are phenomena in modern world. Several environmental factors alone or in combination may contribute to adversely affect reproductive health. Limited environmental (arsenic, selenium, fluoride, lead, cadmium, phthalates, vinclozolin, octylphenol, benzo(A) pyrene, chlorpyrifos), therapeutic (cisplatin, carboplatin, vinblastine and bleomycin), plant-derived (aflatoxin B1, daidzein and genistein) and life style (alcohol and restraint stress) factors were selected in my laboratory to evaluate male reproductive health. The reproductive end points selected by us include relative weights of reproductive organs, serum reproductive hormone levels, daily sperm productive production, epididymal sperm motility, viability (live/death ratio) and density, oxidative status of reproductive tissues, histological alterations in the testis and epididymis, fertility and libido of male Wistar rats exposed to selected chemical and physical stress alone or in combination. In silico studies were also carried out to know the binding affinity of selected environmental pollutants to StAR protein and estrogen receptor α compared to respective natural ligands. Our working hypothesis of stress-inducible suppressed reproduction in rat model is as follows: Rats exposed to stress alone from threshold puberty till adulthood provided clear evidence of altered reproductive functions such as decreased reproductive organ indices, reduction in sperm quantity and deterioration in sperm quality, diminished steroidogenic marker enzyme activities, altered serum hormonal levels, elevated oxidative toxicity in the testes and epididymis, deterioration in testicular architecture and decreased male reproductive efficiency. Additionally, in silico studies suggest the interaction of selected chemicals with StAR, which might result in reduced transport of cholesterol into mitochondria that ultimately affects androgen synthesis in stressed rats. The interactive effect of stresses in inducing oxidative toxicity in testis and epididymis, in decreasing spermatogenesis and in suppressing fertility can be described as additive. Conversely, the decrease in steroidogenesis in stressed rats can be shown as both additive and synergistic. The combination of stresses selected by us may not represent all stresses experienced by humans but such interactions are possible in the general environment and act simultaneously on male reproductive system. Extrapolation of rat data to human is always difficult. However, it should be noted that the doses of chemicals selected in our laboratory are relevant to the doses exposed in the contemporary society.

ADDITIVES IN SPERM CRYOPRESERVATION

Prof Diah Tri Widayati Ir. M.P., Ph.D., IPM

Various additives are used in sperm cryopreservation to increase the viability of frozen sperm in order to increase the fertilization rate. Some determinate additives have been added to sperm diluent, including antioxidants, antibiotics, and stimulants. Cryopreservation involves exposing the sperm cell to a low temperature and a potentially harmful condition, which can lead to the production of reactive oxygen species (ROS) and oxidative damage. Antioxidants play a crucial role in sperm cryopreservation by protecting the sperm cell from oxidative stress, which can occur during the freezing and thawing processes. There are 2 categories of antioxidants, enzymatic antioxidants (for example superoxide dismutase (SOD), catalase, and glutathione peroxidase) and non-enzymatic antioxidants (for example vitamins C and E, glutathione, and selenium). These enzymatic antioxidants convert harmful ROS into less reactive species, neutralizing their damaging effect on spermatozoa. Non-enzymatic antioxidants directly interact with ROS, neutralizing them and preventing their harmful effect on sperm membrane lipids, proteins, and DNA. Several antioxidants commonly used are glutathione (GSH), glutathione disulfide (GSSH), vitamin C, and vitamin E. The addition of antioxidants has been investigated in various studies to assess their potential impact on semen quality after thawing. The specific antioxidant compounds and their concentrations can vary between studies. Several studies have reported that the addition of antioxidants to semen before freezing can help preserve sperm motility. Antioxidants help prevent oxidative damage to sperm cell membranes, thereby preserving their integrity and viability, and help maintain DNA integrity in sperm cells to yield improved post-thaw DNA quality. This can contribute to improved post-thaw membrane integrity and the functionality of sperm cells. Antibiotics are added to cryopreservation media to prevent the growth of bacteria or fungi that could harm the sperm cell, prevent contamination, and reduce the risk of infection during storage. Commonly used antibiotics include penicillin, streptomycin, and gentamicin. Stimulants can be used in sperm cryopreservation to improve sperm quality, enhance the viability of the frozen sperm, and optimize post-thaw recovery. Commonly used stimulants are caffeine and pentoxifylline. Caffeine increases the intracellular cyclic adenosine monophosphate (cAMP) level and improves sperm motility and enhanced capacitation. Pentoxifylline not only increases cAMP levels, but also improves sperm motility, hyperactivation, and sperm-zona pellucida interaction. In this lecture, I will focus on the causes of ROS, the mechanisms involved in sperm damage, and the role of additives in maintaining sperm fertility.

FEMALE INFERTILITY - AN OVERVIEW

Associate Prof Nuguelis Razali MBBS (UM), MObGyn (UM)

Infertility is defined as the failure to achieve pregnancy after 12 months of regular unprotected sexual intercourse. About 85% of infertile couples have an identifiable cause with the most common being ovulatory dysfunction, male factor infertility, and tubal disease. The remaining 15% of infertile couples have “unexplained” infertility. Ovulatory disorders accounts for 25% of infertility while tubal infertility contributes to 11-67% depending on the population studied. Ovulatory disorders are treated with ovulation induction with various pharmacologic agents. Following successful ovulation induction, the women may proceed with timed sexual intercourse or intrauterine insemination (IUI). If these failed or in certain circumstances, ovarian stimulation is performed with the aim of obtaining multiple mature oocytes followed by *in vitro* fertilisation (IVF). Women with unilateral tubal blockage can be treated with IUI. For women with bilateral tubal blockage, the options of surgery to restore tubal patency or IVF can be considered. An important factor that affects fertility is the woman’s age. With increasing age, there is a drastic decrease in the number and quality of oocytes. Therefore, women who are older than 35 should be evaluated earlier i.e., after 6 months of trying. Similarly, older women for example those who are between 38-40 are usually offered IVF as first line treatment instead of IVF or IUI. The aim of this lecture is to discuss the various causes of female infertility and the treatment options available to overcome female infertility.

ORAL PRESENTATION SCHEDULE

Wednesday 18 October 2023			
Oral session 1 (12:00 pm – 12:45 pm) Chairpersons : Prof Arief Boerdiono (*IPB University, Indonesia*) Associate Prof Victor Navarro (*Harvard University, USA*)			
O-1	12:00 pm – 12:15 pm	Dr Nurul Akmal Binti Jamaludin *Management & Science University, Malaysia*	Extracellular vesicles mediated intercellular communication between spermatozoa and oviduct: A new paradigm of spermatozoa – oviduct cross talk
O-2	12:15 pm – 12:30 pm	Dr Hilwah Nora *Universitas Syiah Kuala, Indonesia*	Characteristics of Endometriosis Patients who Underwent Surgery in Dr. Zainoel Abidin General Hospital, Banda Aceh, Indonesia
O-3	12:30 pm – 12:45 pm	Nina Keterina Hashim *Universiti Teknologi MARA, Malaysia*	Impact of Glycine L-Proline Supplementation on Intracellular ROS Levels in Vitrified Mouse Embryos
O-4	12:45 pm – 1:00 pm	Dr Raymond Lim *Universiti Malaya, Malaysia*	Correlation of Multinucleated Embryo and Pregnancy Outcome

Oral session 2 (2:15 pm – 3:30 pm) Chairpersons : Prof Nor Ashikin Mohamed Noor Khan (*UiTM. Malaysia*) Dr Kyaimon Myint (*Universiti Malaya, Malaysia*)			
O-5	2:15 pm – 2:30 pm	Dr Farhanah Mohd Hamim *Universiti Teknologi MARA, Malaysia*	Physical Exercise Improves Sperm Parameters in Leptin-Treated and Normal Sprague-Dawley Rats
O-6	2:30 pm – 2;45 pm	Maria Amir *Universiti Putra Malaysia*	Mitigation of lead acetate induced uterine toxicity in rats using edible bird's nest supplement through inhibition of oxidative stress and chelating activity
O-7	2:45 pm – 3:00 pm	Madhumanti Barman *Universiti Malaya, Malaysia*	Downregulation of SMAD pathway in testes and deterioration of sperm parameters in concomitant methimazole-induced hypothyroid and high fat diet-induced obese mice

O-8	3:00 pm – 3:15 pm	Prof Shailesh D. Ingole *Mumbai Veterinary College, India*	Milk miRNA expression as a potential biomarker for early prediction of mastitis in buffaloes
O-9	3:15 pm – 3:30 pm	Atikah Nur Baity *Universitas Gadjah Mada, Indonesia*	Effect of Storage Periods on Quality of post-Thawed Semen of Bali Cattle

Oral session 3 (3:45 pm – 5:00 pm)

Chairpersons: Prof Diah Tri Widayati (*Universitas Gadjah Mada, Indonesia*)
Dr Khadijeh (Shadi) Gholami (*International Medical University, Malaysia*)

O-10	3:45 pm – 4:00 pm	Nur Athirah Lope Abdul Rashid *Universiti Putra Malaysia*	Slow Freezing versus Vitrification for Cryopreservation of Malaysian Village Chicken (*Gallus domesticus*) Ovarian Tissue
O-11	4:00 pm – 4;15 pm	Nur Erysha Sabrina Jefferi *Universiti Kebangsaan Malaysia*	EVNol SuprabioTM Ameliorates Testicular Steroidogenesis via Reproductive Hormone Regulation in Bisphenol F-Induced Sprague Dawley Rats
O-12	4:15 pm – 4:30 pm	Wafriy Che Ismail *Universiti Teknologi MARA (UiTM) Malaysia*	Altered Embryo Ultrastructure and Cell Organelles in Ovalbumin-induced Allergic Asthma Model
O-13	4:30 pm – 4:45 pm	Nur Amanina Syariff Tan *Universiti Malaya, Malaysia*	Downregulation of spermatogenic proteins and abnormal sperm parameters in Vitamin D deficient obese male mice
O-14	4:45 pm – 5:00 pm	Prof Kishori Battini *Sri Padmavati Mahila Visvavidyalayam (Women's University), India* – ONLINE	Current Perspectives on Potential Effects of Micro and nanoplastics on Male Reproductive Health

Thursday 19 October 2023			
Oral session 4 (11:00 am – 12:45 pm) Chairpersons: Professor Dr Mukhri Hamdan (*Universiti Malaya, Malaysia*) Dr Huma Shahzad (*International Medical University, Malaysia*)			
O-15	11:00 am – 11:15 am	Associate Prof Damayanthi D/ Ifrah Alam Malik *Universiti Teknologi MARA*	Profortil Prevents Some of the Adverse Effects of leptin on Sperm in Rats
O-16	11:15 am – 11:30 am	Dr Irfan Yunianto *Universitas Ahmad Dahlan, Indonesia*	*In vitro* evaluation of ruxolitinib to target JAK/STAT pathway in a microenvironment that mimics ovarian cancer
O-17	11:30 am – 11:45 am	Dr Regina Marhadisony *Universitas Syiah Kuala, Indonesia*	Hysteroscopic-guided removal of trans caesarean Intrauterine Devices in Patients with prior extraction failure in Zainoel Abidin Hospital, Banda Aceh, Indonesia
O-18	11:45 am – 12:00 pm	Syalsa Bella Fitriana *Universitas Gadjah Mada, Indonesia*	Effect of Different Thawing Methods on Frozen Semen Characteristics of Simmental Bull
O-19	12:00 pm – 12:15 pm	Qistina Aisyah Saifull Adli *Universiti Kebangsaan Malaysia*	Efficacy of *Moringa oleifera* (MO) Extract in Treating Male Infertility Associated with Sperm DNA Fragmentation
O-20	12:15 pm – 12:30 pm	Norermi Firzana Alfian/ Prof Satoshi Kishigami *University of Yamanashi, Japan* -ONLINE	Different Response of *In Vivo* and *In Vitro* Fertilized Embryos Against Supplementation of Riboflavin and Pyridoxine During the Preimplantation Period
O-21	12:30 pm – 12:45 pm	Faiza Alam *Universiti Brunei Darussalam* - ONLINE	Unveiling the Impact of Vitamin D: Investigating Sirt1, Antioxidants, and Female Infertility

Oral session 5 (2:15 pm – 4:30 pm)

Chairpersons : Prof Sreenivasula P Reddy (*Sri Venkateswara University, India*)
Dr. Shamsul Azlin B Ahmad Shamsuddin (*Universiti Malaya, Malaysia*)

O-22	2:15 pm – 2:30 pm	Syakiera Amanda *Universiti Teknologi MARA, Malaysia*	Iron Dysregulation Promotes Ferroptosis in Allergic Asthma Pregnant Mouse Model
O-23	2:30 pm – 2:45 pm	Ang Wan Yi Universiti Malaya, Malaysia	*In-vitro* treatment with Vitamin D improves Human Sperm Motility and Protein Expression
O-24	2:45 pm – 3:00 pm	Asma' Afifah Shamhari *Universiti Kebangsaan Malaysia*	Subacute and Sub chronic Exposure of Bisphenol F Induced Estrogen-like Effect in Male Sprague-Dawley Rats
O-25	3:00 pm – 3:15 pm	Husna, A *Universiti Teknologi MARA Malaysia*	Ferroptosis and Intracellular Senescence During Embryonic Development: A Systematic Review
O-26	3:15 pm – 3:30 pm	Farah NS *Universiti Malaya, Malaysia*	Protective role of *Cocos nucifera* L. water on BPA-mediated oxidative stress and reproductive health damage in male rats
O-27	3:30 pm – 3:45 pm	Datu Agasi Mohd Kamal / Associate Prof Mohd Helmy Mokhtar *Universiti Kebangsaan Malaysia*	Kelulut Honey Alleviates Ovarian Steroidogenic Enzymes Profiles in Letrozole-Induced Polycystic Ovary Syndrome Rats
O-28	3:45 pm – 4;00 pm	Amirah Baharin *Universiti Malaya, Malaysia*	Bioactivity and Pharmaceutical Potential of Phoenix dactylifera on Morphine-Induced Toxicity in Male Rats: An Animal Model Study
O-29	4:00 pm – 4:15 pm	Razia Sardar *Universiti Teknologi MARA, Malaysia*	Effects of Glutathione on Sperm Count and Sperm Morphology in STZ-Induced Diabetic Mice
O-30	4:15 pm – 4:30 pm	Selvakumar Mararajah/ Dr Nelli Giribabu *Universiti Malaya, Malaysia*	Protective effect of Stigmasterol against hydrogen peroxide-induced oxidative stress in mouse testes and human sperm: Experimental and computational approach

POSTER PRESENTATION SCHEDULE

Wednesday 18 October 2023 12:45 pm – 2:15 pm		
Judges: (i) Associate Prof Victor Navarro (*Harvard University, USA*) (ii) Associate Prof Hoe See Ziau (*Universiti Malaya, Malaysia*) (iii) Prof Diah Tri Widayati (*Universitas Gadjah Mada, Indonesia*) (iv) Dr Kumar Seluakumaran (*Universiti Malaya, Malaysia*)		
P-1	Nur Afizah Yusoff *Universiti Kebangsaan Malaysia*	Oxidative Stress Evaluation in Lineage-Related Toxicity of *In-Utero* Hydroquinone-exposed Hematopoeitic/Stem Progenator Cells of Maternal Mice
P-2	Ainul Bahiyah *Universiti Sains Malaysia*	Bee Bread Ameliorates Steroidogenesis and Spermatogenesis Impairment in High-Fat Diet-Induced-Obesity Rat Model
P-3	Noor Asyikin Suaidi *Universiti Malaya, Malaysia*	Xylene Exposure during Early Gestation Impairs Feto-placental Dynamics and Induces Fetal Skeletal and Head Variations in Sprague-Dawley Rats
P-4	Dr Nor Shazlina M. S *University Putra Malaysia*	Oocyte Grading and Morphometry in the Indigenous Kedah-Kelantan Cattle Breed for Enhancement of Beef Cattle Genetics in Malaysia
P-5	Nurul Hamirah Kamsan *Manipal Melaka University College, Malaysia*	Tocotrienol-Rich Fraction Modulates the Cell Cycle Signalling Pathway Genes in Preimplantation Embryos of Nicotine-Induced Oxidative Stress Model
P-6	Syawany Wahid *Management and Science University, Malaysia*	Effects of Kombucha Extract Towards Metabolic Changes in Induced-Letrozole Polycystic Ovarian Syndrome (PCOS) in Rats: A Preliminary Study
P-7	Maisarah Nadhirah *Universiti Malaya, Malaysia*	Effect of Mature Coconut Water Supplement on the Reproductive Organ of BPA-Treated Female Rats
P-8	Nur Faizah Nadia MZ *Universiti Malaya, Malaysia*	The Potential of Mature Coconut Water as a Dietary Supplement in Alleviating Oxidative Stress and Enhancing Sperm Quality
P-9	Izatus Shima Taib *Universiti Kebangsaan Malaysia*	Palm Oil Tocotrienol Rich Fraction Ameliorate the Estrogen-Like Effects Induced by Bisphenol F in Testicular Tissue of Sprague Dawley Rats

P-10	Ain NK *Universiti Malaya, Malaysia*	The impact of preservation temperature and exposure time on Dorper ram sperm quality
P-11	Anisa-Annur *Universiti Kuala Lumpur Royal College of Medicine. Malaysia*	The effect of Exogenous Coenzyme Q10 Supplementation on Mitochondrial Intensity of Vitrified Mouse Embryo
P-12	Siti Khadijah Idris *Universiti Malaya, Malaysia*	Conventional Incubator vs. Time-lapsed Incubator Culture System: Comparison of Embryo Development and Subsequent Pregnancy Outcome
P-13	Nurul Izzaty Najwa Aziz *Universiti Malaya, Malaysia*	Fertilizing Capability of Low to Borderline Sperm Concentration in Conventional In Vitro Fertilization (IVF)
P-14	Selvakumar Mararajah *Universiti Malaya, Malaysia*	Amelioration of hydrogen peroxide (H2O2)-induced oxidative stress in testes and sperm of mice by *Chlorophytum borivilianum* aqueous root extract
P-15	Aqila-Akmal Mohammad Kamal *Universiti Teknologi MARA, Malaysia*	Effect of Maternal Bisphenol A Exposure Below NoObserved-Adverse-Effect-Level (NOAEL) on Apoptosis in BALB/c Mouse Embryos.
P-16	Sharifah Mahfudzah Syed Mafdzot *Universiti Malaya, Malaysia*	Comparison of Day-3 and Day-5 Embryo Transfer from Fresh and Frozen Cycles in determination of the IVF Pregnancy Outcomes
P-17	Haizal Danial Esah *Universiti Malaya, Malaysia*	Effect of Dietary Supplementation of Mature Coconut Water on Semen Cryopreservation of Boer Bucks
P-18	Dr Ima Indirayani *Universitas Syiah Kuala, Indonesia*	Characteristics and Quality of Life Among Menopausal Women in Banda Aceh, Indonesia
P-19	Budi Purwo Widiarso *Polytechnic of Agricultural Development Yogyakarta-Magelang, Indonesia*	Ketapang Leaf (*Terminalia catappa L*) Extract Supplementation Increases the Expansion of Cumulus Cells in Beef Cattle Oocyte Maturation *In-Vitro*
P-20	Dr Nurul Fadhliati Maulida *Universitas Syiah Kuala, Indonesi*a	Complete Androgen Insensitivity Syndrome (CAIS): A Rare Case Report

ABSTRACTS FOR ORAL PRESENTATION

O-1 Extracellular vesicles mediated intercellular communication between spermatozoa and oviduct: A new paradigm of spermatozoa – oviduct cross talk

Nurul Akmal Jamaludin

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The interaction of gametes and embryo with the maternal environment has a crucial impact on gametes maturation, embryonic development and subsequent pregnancy success. Recent studies have recognised extracellular vehicles (EVs) as potent vehicles for intercellular communication in wide variety of physiological systems. EVs range from 30-1000nm and are composed of a lipid bilayer containing transmembrane proteins and enclosing soluble proteins, and genetic materials. Once secreted by their cell of origin, it is thought that recipient cells are able to target and bind EVs via their surface proteins thus mediating communication between cell types. Several studies had demonstrated the secretion of EVs by female reproductive tract in response to the arrival of gametes and embryo. However, so far study that looking at the role of EVs during spermatozoa-oviduct interaction is still very limited. Therefore, the main aim for this present study is to investigate the characteristics and role of EVs in the modulation of successful spermatozoa-oviductal epithelial cell (OEC) interactions. This study also sought to investigate the different characteristics and role of EVs-derived microRNAs (EVs-miRNAs) secreted during spermatozoa-OEC interaction. This was achieved by the determination of the different EVs-miRNAs secreted by OEC in the presence of spermatozoa in a simple *in vitro* model. The results showed that the interaction between spermatozoa and oviduct will alter the EVs-miRNA expression. Further clarification of the role of EVs-miRNAs in reproductive processes will enable us to target mechanisms to increase *in vivo* conception rates and enhance assisted reproductive technologies.

Keywords: EVs, miRNA, Spermatozoa, Oviduct Epithelial Cells

O-2 Characteristics of Endometriosis Patients who Underwent Surgery in Dr. Zainoel Abidin General Hospital, Banda Aceh, Indonesia

Nurul Fadhliati Maulida, Rajuddin, Hilwah Nora, Rusnaidi, Ima Indirayani

Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

Endometriosis is an estrogen-dependent disease that can cause pain which interfere daily activity and infertility characterized by the presence of the endometrium (glandular cells and stroma) outside the uterine cavity. It is a chronic inflammatory disease with three common types namely ovarian endometriosis, peritoneal endometriosis, and deep infiltrating endometriosis. This study aimed to describe the characteristics of patients with endometriosis who underwent surgery in Dr. Zainoel Abidin General Hospital Banda Aceh from 1 September 2022 to 30 September 2023. The results are depicted in distribution and frequency characteristics tables. This is a descriptive retrospective study, using medical record data of endometriosis patients in Dr. Zainoel Abidin General Hospital Banda Aceh which were confirmed by histopathological examination. Result: There were 43 cases of endometriosis that were confirmed by histopathological examination in Obstetrics and Gynecology wards, Dr. Zainoel Abidin General Hospital Banda Aceh from 1 September 2022 to 30 September 2023 who underwent surgery. Endometriosis cases were most often seen among women aged 20-35 (53,48 %), 32 nullipara patients (74,41%), 35 patients with dysmenorrhea (81,39%), 36 patients with ovarian endometriosis type (83,72%), 17 patients with stage IV of rASRM classification (39,53%). Adenomyosis were seen in 10 patients (23,25%). There were 23 patients (53,48%) received hormonal therapy before undergoing surgery and 20 patients (46,51%) had never received hormonal therapy in which 20 patients (46,51%) underwent laparoscopy surgery, while 23 patients (53,48%) underwent laparotomy surgery. The mean of Ca-125 level was 127,22. There were strong correlation between CA-125 level with endometriosis stage ($P<0.05$; $R=0,501$), type of surgery ($P<0.05$; $R=0,156$), and fertility ($P<0.05$; $R=0,257$). Conclusion: Epidemiological information about characteristic of endometriosis patients may be helpful in identifying groups with specific clinical courses that potentially suggesting novel approaches to early detection and for surgical and systemic treatment.

Keywords: Endometriosis, Classification, Infertility

O-3 Impact of Glycine L-Proline Supplementation on Intracellular ROS Levels in Vitrified Mouse Embryos

Nina Keterina Hashim[1,2], Mimi Sophia Sarbandi[1,3], Nor Ashikin Mohamed Noor Khan[1], Zolkapli Eshak[4]

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Vitrification of embryos is an important procedure in assisted reproductive technology. Although important, *in vitro* culture and vitrification impose stress and expose embryos to reactive oxygen species (ROS). Since embryo culture conditions influence the course of development, the inclusion of ROS protective agents in the culture media is crucial. Both glycine and L-proline have demonstrated protective roles in scavenging ROS. This study investigated the extent to which the addition of glycine and L-proline to embryo culture media can attenuate ROS -induced damage to vitrified embryos. Embryos at the 2-cell stage were cultured *in vitro* with and without glycine-L-proline addition in both non-vitrified and vitrified groups until the blastocyst stage. Embryos that had developed to the 8-cell stage were immunofluorescent stained for ROS. The study showed that the percentage of blastocysts in media with added glycine-L-proline was higher in both the non-vitrified (92.0% vs. 58.7%) and the vitrified group (86.3% vs. 84.3%), than in media without added glycine-L-proline. Mouse embryos cultured in glycine-L-proline culture media also showed a significant decrease in ROS intensity in both the non-vitrified group [(22.24 vs 27.69) pixels 10^5] and the vitrified group [(32.85 vs 40.21) pixels 10^5] compared to media without glycine-L-proline addition ($P < 0.001$). In conclusion, glycine-L-proline media supplementation is a novel alternative to counteract the effects of ROS overproduction during *in vitro* culture and vitrification.

Keywords: Mouse embryos, Vitrification, ROS, Amino acid, Glycine, L-proline.

O-4 Correlation of Multinucleated Embryo and Pregnancy Outcome

Tan Siew Yin, Nuguelis Razali, Raymond Lim. Siti Khadijah Idris, Ruhaima Ramli, Mukhri Hamdan

Dept of Obstetrics & Gynecology, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia

Single embryo transfer is increasingly done in practice to avoid multiple pregnancy. Therefore, it is important to ensure the embryo with the best pregnancy outcome is selected. Although new criteria involving genomic, transcriptomic, and proteomic have been developed to predict embryo outcome, morphology of the embryo remains as the main indicator because it is easy to implement and less controversial. To study the correlation of multinucleated embryos and pregnancy outcomes. Method: This is a retrospective review of a total of 411 embryos from 225 women undergoing their first IVF or ICSI cycle in University Malaya Medical Centre, Kuala Lumpur between January 2019 and October 2021. Multinucleation was defined as the presence of more than one nucleus within at least one of the blastomeres of a 2-cell embryo and a time-lapse imaging system was used to observe embryo development (Embryoscope; Unisense Fertilitech). Group allocation was based on the presence or absence of multinucleation on day 2 after egg retrieval. Embryos with evidence of one or more blastomere having multinucleation were included in the studied group (n = 67 embryos). Embryos without any blastomere exhibiting multinucleation were included in the control group (n = 344 embryos). Results: The data were based on 225 transfers of 411 embryos consisting of 16.3% (n =67) from studied group and 83.7% (n=344) from the control group. The biochemical pregnancy rate and implantation rate is lower in the studied group compared to the control group although not statistically significant (Table 1). Conclusion: Multinucleated embryos reduce the implantation rate and clinical pregnancy rate although not statistically significant.

Table 1

	Study n=67	Control n=344	P Value
Biochemical Pregnancy Rate	19/67 = 28.36	112/344 = 32.56%	0.500
Implantation Rate	17/67 = 25.37%	95/344 = 27.61%	0.706

Keywords: Multinucleated Embryo, Pregnancy Outcomes

O-5 Physical Exercise Improves Sperm Parameters in Leptin-Treated and Normal Sprague-Dawley Rats

Farhanah Mohd Hamim[1], Fayez Almabhouh[1,2], Ifrah Alam Malik[1], Suzanna Daud[3,4], Damayanthi Durairajanayagam[1], Harbindar Jeet Singh[1]

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The effects of treadmill running exercise on sperm count and morphology, reproductive organ weight, and testicular antioxidant enzyme activity in leptin-treated Sprague-Dawley (SD) rats were investigated. Twelve-week-old male SD rats were randomized into four groups (n=8 per group): control (G1), leptin-treated (G2), exercise+leptin-treated (G3), and exercise-only (G4). Controls were given 0.1ml of 0.9% saline, while leptin-treated rats were administered 60µg/kg BW of leptin intraperitoneally for 42 days. Rats in G3 and G4 were exercised four times weekly over a period of 42 days. Their exercise regimen comprised 30 minutes of continuous running on a treadmill (0.5m/s speed, 5° incline). The rats were euthanised on day 43, and total sperm count, fraction of sperm with abnormal morphology, and weights of testes and epididymides were recorded. Testicular catalase and glutathione peroxidase activities were measured. Data were analysed using ANOVA with post-hoc analysis. G2 rats had lower total sperm count and a higher fraction of sperm with abnormal morphology compared to those in the other three groups. Conversely, higher sperm count and lower fraction of sperm with abnormal morphology were observed in G3 rats than in G2 rats. Interestingly, sperm count and morphology were better in G4 rats than in G1 rats. No significant differences were observed in the remaining parameters between the four groups. Regular physical exercise for 42 days was able to improve sperm count and morphology in both leptin-treated and saline-treated Sprague-Dawley rats, suggesting that regular physical exercise is beneficial for male reproductive health.

Keywords: Treadmill exercise, Sperm count, Sperm morphology, Testicular & Epididymal weight, Catalase, Glutathione peroxidase, Male Reproductive Health

O-6 Mitigation of Lead Acetate induced Uterine Toxicity in Rats using Edible Bird's Nest Supplement through Inhibition of Oxidative stress and Chelating activity

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Edible bird's nest (EBN) possesses fertility-enhancing attributes, due to its potentially distinctive composition, which could help to counteract the toxic effects of lead acetate (PbA). PbA is a hazardous chemical that adversely affects female reproduction. The objective of the study was to assess how well EBN pretreatment works to reduce heavy metal, such as PbA toxicity. Five groups (six in each group) of Sprague-Dawley rats were treated to PbA for eight weeks. G1 as control group, G2 given PbA at 10 mg/kg bwt, G3 given PbA and EBN at 60 mg/kg, while G4 and G5 were at 90 and 120 mg/kg, respectively. After treatment, the rats were bred, and on day 8 of pregnancy (pd8), all rats were slaughtered, and their uteri inspected for embryo implantation rate (EIR). Blood samples were taken for oxidative stress biomarker testing. Uterine tissues underwent immunohistochemistry for metallothionein (MT), histology. EBN supplementation revealed augmented fertility by counteracting the adverse effects of PbA. Groups treated with EBN showed increased luminal and glandular epithelium growth in the uterus, higher EIR, accompanied by increased blood profiles of the antioxidative biomarker (SOD) and decreased (TBARS) activity. Likewise, MT expression in uterine tissues was found to be significantly reduced with with higher doses of EBN supplements. Lowered MT expressions in uterine tissues might reflect chelating role of EBN resulting in reduction of PbA accumulation. In conclusion, EBN appeared to protect the uterine function against PbA toxicity through modulation of redox balance, promoting cell growth, proliferation and potential chelating role.

*Keywords***:** Lead acetate toxicity; Edible bird's nest; Metallothionein; Steroid hormones; Oxidative stress biomarkers

O-7 Downregulation of SMAD Pathway in Testes and Deterioration of Sperm parameters in Concomitant Methimazole-induced Hypothyroid and High Fat Diet-induced Obese Mice

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Infertility, a common disease, affecting nearly 48 million (15%) couples worldwide and can be attributed to male and female factors. Studies have found that hypothyroidism and obesity could adversely affect male fertility, however the concomitant effects of both hypothyroidism and obesity on male fertility are unknown. Objectives: To investigate the mechanisms underlying hypothyroidism and obesity condition on male fertility via assessing the SMAD pathway and spermatogenesis in the testes. Methods: ICR adult male mice were divided into five groups i.e., normal control, hypothyroid, obese, hypothyroidism with obesity, and hypothyroidism with obesity supplemented with thyroxine for 28 days. Hypothyroidism with obesity were confirmed by measuring serum thyroxine and leptin levels. At the end of the treatment, mice were sacrificed and testis were collected and sperm were harvested from cauda epididymis for analysis. Results: Body weight and serum leptin levels significantly increased in obese mice and in mice with hypothyroid with obesity while serum thyroxine levels are low in hypothyroid and hypothyroid with obesity groups. SMAD pathway is downregulated in testes of hypothyroid with obesity mice as evidenced by decreased levels of SMAD2 and connexin-43 proteins. Meanwhile, in these mice, there is increased number of sperm with abnormal morphology, vitality and HOS test. The number of sperm with DNA fragmentation also increased. These parameters were reversed by thyroxine treatment. Conclusion: Reduced spermatogenesis and abnormal sperm parameters in hypothyroidism with obesity condition involve the SMAD pathway in testes which can be improved by thyroxine, suggesting the role of thyroxine in this condition.

Keywords: Male Infertility, Hypothyroid, Obesity, Thyroxine, Sperm Quality

O-8 Milk miRNA expression as a Potential Biomarker for Early Prediction of Mastitis in Buffaloes

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Early disease detection is crucial for reducing the economic losses and animal welfare impact of bovine mastitis worldwide. Bovine mastitis is a prevalent and costly disease affecting dairy industries globally. By identifying and treating mastitis at its early stages, farmers can minimize production losses, reduce the use of antibiotics, and ensure overall animal health and welfare. Thus, the study is focused on analysing the expression levels of two microRNAs (miR-146a and miR-383) in buffalo milk samples with different mastitis conditions. Thirty buffalo milk samples were divided into three groups based on CMT as normal, sub-clinical mastitis, and clinical mastitis were collected from the Mumbai region. SCC evaluation showed a significant difference ($p<0.000$) between the three groups. The miR-146a and miR-383 expression was increased in the milk of buffaloes with mastitis. Moreover, clinical mastitis showed a greater expression level of miR-146a and miR-383 than sub-clinical mastitis. There was a significant difference ($p<0.000$) in miR-146a and miR-383 ($p<0.001$) expression between normal, subclinical, and clinical mastitis milk of buffaloes. The study found a positive correlation between SCC and miR-146a and miR-383 expression levels in normal, sub-clinical, and clinical mastitis milk samples. This suggests these microRNAs are associated with inflammation and could serve as valuable prognostic biomarkers for early mastitis detection in buffaloes, especially when SCC is below two lakhs and CMT is negative. In conclusion, the study highlights the potential of miR-146a and miR-383 as biomarkers for detecting mastitis in buffaloes and suggests their relevance in differentiating between normal and mastitis conditions.

Keywords: Milk, Mastitis, miRNA, Buffaloes

O-9 Effect of Storage Periods on Quality of Post-Thawed Semen of Bali Cattle

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This research aimed to determine the effect of storage periods in liquid nitrogen on quality of post-thawed semen of Bali cattle. This research was conducted at the Laboratory of Animal Physiology and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada. Ninety semen straws produced during December 2022 (8 Month), February 2023 (6 Month), and May 2023 (3 Months) from one bull were used in this research. The samples straw was divided into 3 group; each group consist 30 semen straws as long as 3-, 6-, and 8-month storage in liquid nitrogen. The quality of thawed semen included motility, viability, abnormality, and membrane integrity were observed. The collected data was analysed using one-way analysis variance (ANOVA) with IBM SPSS Statistic 27. The storage of 3 months (45.61±0.61%), 6 months (44.78±0.67%), and 8 months (44.56±0.44%) did not show the significant difference ($P>0.05$) on motility. However, the storage had a significant effect on thawed semen viability ($P<0.05$). Whereas, the viability of thawed semen between storage of the 3 months (73.25±0.89%) and 8 months (73.13±1.42%) was not significantly different ($P>0.05$). The abnormality and plasma membrane integrity of thawed semen was significantly different ($P<0.05$) with storage on the 3 months (9.76±0.50%), 6 months (12.67±0.40%), and 8 months (15.85±0.73%). On the other hand, the plasma membrane integrity was not significantly different ($P>0.05$) between storage of 3 months (73.72±0.63^{a} %) and 6 months (70.27±1.28^{a} %), also storage of 6 and storage 8 months (69.17±1.32^{a} %). Conclusions: *Based on the result* above, it *could be concluded* that storage had effect on the viability, membrane integrity and abnormality of post thawed semen of Bali cattle. The best results were obtained at 3 months of storage in liquid nitrogen.

Keywords: Bali Cattle, Spermatozoa, Storage periods, Liquid Nitrogen, Semen Quality

O-10 Slow Freezing versus Vitrification for Cryopreservation of Malaysian Village Chicken (*Gallus Domesticus*) Ovarian Tissue

Nur Athirah Lope Abdul Rashid[1], Mazlina Mazlan[2], Intan Shameha Abdul Razak[1] and Awang Hazmi Awang Junaidi[1]

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Ovarian tissue cryopreservation is a tool for the storage of female germplasm for conservation and therapeutic applications. In birds, the reproductive potential of the cryopreserved tissue can be revived via orthotopic transplantation, followed by the artificial insemination of the male gametes, which results in the production of donor-derived offspring. This study aimed to compare the efficiency of two cryopreservation techniques; slow freezing and vitrification, on the Malaysian Village Chicken (MVC) ovarian tissue. Fresh ovaries from MVC chicks (age: <1 week old) were cryopreserved using either slow freezing or vitrification technique (n=10/technique). The tissues were stored in liquid nitrogen (-196°C) for up to 3 months. Samplings were performed at 1- and 3 months' post-cryopreservation (n=5/sampling/technique), where the cryopreserved tissues were subjected to macroscopic and microscopic evaluations. Gross weight and diameter of the tissue samples were recorded. The germinal epithelium integrity and vacuolation of the ovarian tissue were scored, and the number of follicles and cortex thickness were measured. The viability of the tissue was also assessed using the trypan blue exclusion assay. Results showed the vitrified samples were shrunken and the weight reduced over time ($p<0.05$). The 1-month vitrification samples demonstrated higher viability (~40%; $p<0.05$) than the slow freezing samples (~20%). Microscopically, the germinal epithelium integrity and number of follicles significantly reduced ($p<0.05$), while the vacuolization of the ovarian tissue significantly increased ($p<0.05$) over time, in both techniques. In conclusion, the vitrification technique produced slightly better outcomes and could be further manipulated for cryopreservation of MVC ovarian tissue.

Keywords: Cryopreservation, Ovarian tissue, Birds, Slow freezing, Vitrification

O-11 EVNol Suprabio™ Ameliorates the Testicular Steroidogenesis via Reproductive Hormone Regulation in Bisphenol F-Induced Sprague Dawley Rats

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Bisphenol F (BPF) is one of the endocrine disruptors which causes reproductive hormonal changes. EVNol SupraBio™ (EVNol) is a tocotrienol-rich supplement that is well-known for its anti-inflammatory properties. However, effects of EVNol towards BPF-induced changes on testicular steroidogenesis via inflammatory pathways remain unclear. Therefore, present study was done to explore the effects of EVNol on hormonal regulation in testis induced by BPF by assessing reproductive hormone analysis (plasma), inflammatory and antioxidant status of Leydig cells as well as sperm characteristics. Forty male Sprague-Dawley rats (weighed between 220-250g) were randomly classified to five groups which are control group (1mg/kg corn oil), EV100 (100mg/kg EVNol), BPF (10 mg/kg BPF), BE50 (50 mg/kg EVNol + BPF) and BE100 (EV100+ BPF). Treatments were administered daily via oral gavage for 35 days and (BE) rats were given EVNol 30 minutes prior to administration of BPF. Current results showed that sperm motility in EV100 group was significantly increased compared to BPF group. EVNol reduced inflammation rate as level of arachidonic acid (AA) was significantly higher in EV100 and BE100 groups whereas PGE-2 was significantly reduced in EV100 group compared to BPF group ($p<0.05$). Significant increase of GSH in EV100 group showed improved antioxidant capacity compared to BPF group. Hormone analysis showed significantly higher levels of cholesterol and testosterone in EV100 group compared to BPF group. EV100 and BE100 groups demonstrated significantly increased pregnenolone and reduced estradiol levels compared to BPF groups ($p<0.05$). Conclusively, 100 mg/kg of EVNol ameliorates testicular steroidogenesis in BPF-induced rats by attenuating inflammation.

Keywords: Bisphenol F, BPA analogue, Male Reproductive system, Steroidogenesis, EVNol SupraBio™, Vitamin E, tocotrienol.

O-12 Altered Embryo Ultrastructure and Cell Organelles in Ovalbumin-induced Allergic Asthma Model

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There are emerging evidence linking poor preimplantation embryo development with allergic asthma. Previously, we reported impaired embryonic-cleavage division, -compaction and -cavitation activity in ovalbumin (OVA)-induced allergic asthma model with activated inflammatory-oxidative stress as the mechanism. However, the ultrastructural features of embryonic organelles of this model remains elusive. In this study, the effect of allergic asthma on the parameters was determined. Female BALB/c (5-week-old, 20–25 g) were divided into OVA-induced allergic asthma and control groups. Treated animals were induced with allergic asthma through a series of sensitization, challenge followed by metacholine (MCh) test. Control animals were treated similarly using PBS. To induce follicular maturation and ovulation, animals were superovulated prior to mating with fertile males. Following euthanization, 2-cell embryos were retrieved, fixed, and dehydrated. The samples were trimmed, sectioned, and stained using ultramicrotome prior to viewing under the transmission electron microscope (TEM) imaging. Irregular blastomere membrane, low mitochondria count, thickened zona pellucida, damaged cytoplasm and absence of lipid droplets in 2-cell embryos were evident in the allergic asthma group. Theoretically, biogenesis of lipid droplets happens as general protective mechanism against stress. Hence, absence of lipid droplets causes redox imbalance leading to increase cell susceptibility to stress. These features are consistent with iron dysregulation and redox imbalance which may lead to cellular dysfunction. Findings indicate that altered ultrastructure of embryonic organelles and cellular disintegrity might be due to lipid- and/or iron-dysregulation. Moreover, further elucidation into iron metabolism and lipid signalling pathway will explain molecular mechanisms behind allergic asthma during pregnancy.

Keywords: Allergic Asthma, Cell Ultrastructure, Organelles, Iron regulation, Lipid peroxidation Transmission Electron Microscope (TEM)

O-13 Downregulation of Spermatogenic proteins and Abnormal Sperm parameters in Vitamin D deficient Obese male mice

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Obesity has adverse effect on male reproduction. In obesity, vitamin D levels are found to be low which raises concern that obesity-related vitamin D deficiency have a negative impact on male reproductive function, in view that vitamin D receptors are found in the seminiferous tubules and in sperm. Therefore, the aims of this study are to investigate the effect of vitamin D deficiency and obesity on male reproductive parameters i.e., spermatogenesis and sperm function. Method: Adult ICR male mice (age 6-8 weeks, n=48) were divided into four groups: standard diet with 2000 IU/kg vitamin D (SD/VD+), standard diet without vitamin D (SD/VD-), high-fat diet with 2000 IU/kg vitamin D (HFD/VD+), and high-fat diet without vitamin D (HFD/VD-). Following 12 weeks of treatment, mice were sacrificed and testes were harvested and epididymal sperm were retrieved. Serum levels of testosterone, LH, FSH and vitamin D were measured. Results: In obese mice with Vitamin D deficiency, sperm count, motility, vitality and HOS tail-coil sperm decreased and sperm with DNA fragmentation increased. Additionally, there is decrease in sperm mitochondrial markers (ATPB, COX IV, TOMM20, JAM-A). Meanwhile, obese mice with Vitamin D deficiency also have decreased levels of vitamin D signalling proteins (VDR, RXR α/β/γ, Vdr, Cyp24a1, and Cyp27b1), blood-testis barrier proteins (ZO2, Vimentin, Ocln, Cdhd2, and Vim) and germ cell marker proteins (GATA-4, JAM-B, SMAD5, Sirt1, Sox9, and Zbtb16) in testes. These changes were ameliorated by Vitamin D supplementation ($p<0.05$). Conclusion: Vitamin D is essential for spermatogenesis and normal sperm parameters, thus could potentially be used as a supplement to improve male fertility in obese conditions.

Keywords: Vitamin D Supplementation, Obesity, Spermatogenesis

O-14 Current Perspectives on Potential Effects of Micro and Nanoplastics on Male Reproductive Health

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Infertility among the population at reproductive age is one of the serious ongoing problems all over the world. Though the root causes are yet to be established, accumulation of data indicated that the exposure to environmental toxicants could be one of the possible factors. Among the environmental toxicants, exposure of humans to the breakdown products of plastic wastes into micro- and nano-plastics is unavoidable. The data up to 2019 indicated that the plastic production exceeded almost 398 million tons worldwide and due to improper management, their breakdown products micro- and nano-plastics enter into the food chain of humans via food and water. Published reports have shown that micro- and nano-plastics causes health hazards in humans including male reproductive abnormalities. In this regard, it is important to consider the potential threats of micro- and nano-plastics on reproductive health. The present paper deals with three aspects. The first aspect provides comprehensive data about the toxic impact of micro and nano-plastics on male reproductive health. The second aspect deals with the mechanisms underlying micro- and nano-plastic mediated reproductive toxicity in males. The final aspect deals with the current gaps and challenges that should be addressed to overcome toxicity of micro- and nano-plastics. To summate, the comprehensive data covered in this review could be helpful for the young researchers to design experiments and develop therapeutic strategies in animal models intoxicated with micro- and nano-plastics.

Keywords: Micro and Nanoplastics, Male Reproductive Health

O-15 Profortil Prevents Some of the Adverse Effects of Leptin on Sperm in Rats

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Oxidative stress is implicated in leptin-induced adverse effects on sperm and testes. This study, therefore, investigated the effects of Profortil®, a concoction of several different antioxidants, on leptin-induced effects on sperm in rats. Methods: Adult Sprague-Dawley rats were divided into 4 groups and given either 0.1ml normal saline (Control) or 60µg/kg/day of leptin or 50mg/kg/day of Profortil® along with leptin or Profortil® with normal saline. Normal saline and leptin were given daily via an intraperitoneal injection for 2 weeks. Profortil® treatment was commenced 1 week before as pretreatment, followed by 2 weeks of treatment concurrently with leptin and normal saline via oral gavage. At the end of the treatment, total sperm count, percentage of sperm with abnormal morphology, levels of 8-OHdG, testicular cell apoptosis and sperm DNA integrity were determined. Results: Profortil improved the total sperm count and 8-OHdG levels in leptin-treated rats but had no effect on sperm morphology. Testicular cell apoptosis was higher in leptin and Profortil and leptin treated groups compared to that in control and Profortil only treated groups. Profortil, however, was not able to prevent the negative effect of leptin on sperm DNA integrity. Conclusion: Treatment with Profortil® had positive effects on some of the sperm and testicular tissue parameters in leptin-treated rats. Further studies with higher doses of Profortil® or a longer duration of treatment, are required to confirm the exact benefits of Profortil on sperm.

Keywords: Leptin, Profortil®, Oxidative Stress, Male Reproductive System, Infertility

O-16 *In vitro* evaluation of ruxolitinib to target JAK/STAT Pathway in a Microenvironment that mimics Ovarian Cancer

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Ovarian cancer (OC) is the most lethal gynaecologic malignancy in the world. Due to absence of specific symptoms, patients with OC are commonly diagnosed at the late stage of the disease when the survival rate is low. Furthermore, patients with advanced OC develop ascitic fluid, which is associated with poor prognosis. The components in ascitic fluid promote tumour progression and chemoresistance via activation of multiple cell signalling routes, including the Janus Kinase (JAK) and Signal Transducer and Activator of Transcription (STAT) pathway. This study aimed to evaluate the effects of ruxolitinib, a JAK1/2 inhibitor, in the monolayer and 3D cell cluster models of three OC cell lines: SKOV3, ID8 and OV90, in the absence or presence of ascitic fluid that mimics the microenvironment of OC. Methods: All cell lines were cultured in monolayer and 3D cell cluster without or with 10% ascitic fluid from the patient. Ruxolitinib treatment was conducted for 72 hours prior to analysing cellular metabolism and proliferation using Alamar Blue and CyQUANT NF assays, respectively. Apoptotic cells were estimated using Annexin V-FITC/PI assay and flow cytometry. A human phospho-kinase proteomic array was utilised to investigate protein phosphorylation after ruxolitinib treatment without or with ascitic fluid on OV90 cells in both culture models. Results: Ruxolitinib reduced cell metabolism in monolayer and 3D cluster models, and these effects were attenuated by ascitic fluid. However, ruxolitinib did not significantly reduce cell proliferation in 3D cluster models. Ruxolitinib treatment increased apoptosis in OC cells cultured in monolayer in a cell-dependent fashion, but not in 3D cluster models. There was a global reduction in protein phosphorylation in OV90 cells treated with ruxolitinib in both culture models, and ascitic fluid mitigated this reduction. Conclusion**:** This study demonstrated the efficacy of ruxolitinib in reducing OC cell viability and global protein phosphorylation in OC cells cultured in monolayer. However, these effects were strongly attenuated in 3D cell cluster models, especially in the presence of ascitic fluid. Thus, the potential use of ruxolitinib as a therapy requires further investigation in models that more closely mimic the tumour microenvironment in OC.

Keywords: Ovarian Cancer, Ascitic Fluid, JAK/STAT, Ruxolitinib, Monolayer, 3D Clusters, Protein Phosphorylation

O-17 Hysteroscopic-guided removal of Transcaesarean Intrauterine Devices in Patients with prior extraction failure in Zainoel Abidin Hospital, Banda Aceh, Indonesia

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The use of intrauterine devices (IUDs), which have few and tolerable adverse effects, is a safe and effective method of long-term reversible contraception. A higher percentage of IUD use six months later was linked to IUD placement during caesarean delivery. Women having an IUD installed via transcaesarean delivery should get advice regarding the chance of IUD expulsion and the potential for hidden IUD strings. An intrauterine device is simple to remove if the string is visible during a speculum exam. The task gets more challenging when the string is hidden. Methods: The in-patient and out-patient medical records of all patients hospitalized for hysteroscopic-guided intrauterine device removal at Zainoel Abidin Hospital in Banda Aceh between 2021 and 2022. Demographic information, an intraoperative record, and information about the post-operative course and result were acquired. Prior IUD removal attempts were also documented. The whole operation time, IUD type removed, operative findings, and any issues experienced were all documented. Results: There were a total of fifteen patients, all of them were of reproductive age. Almost all of them were multigravida. The most frequent justifications for IUD removal were pain, spotting, wanting to get pregnant, and the device's use-by date. The polyclinic or a private practice has previously tried to remove IUDs on all of the women. The majority of the patients experienced typical post-operative results with no readmissions. Conclusion: When ultrasound-guided removal is unsuccessful, hysteroscopic guidance is a better management choice. Complications and major operations that weren't essential were averted. There haven't been any significant issues with the procedure or re-admissions in the 1 year of experience. After ultrasound-guided removal failed, it was found that hysteroscopic removal of the IUD was a successful alternative with a low risk of complications.

Keywords: Hysteroscopy, Intrauterine device removal, Transcaesarean Intrauterine Device, Retained Intrauterine Device

O-18 Effect of Different Thawing Methods on Frozen Semen Characteristics of Simmental Bull

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Thawing technique of frozen semen for artificial insemination (AI) has a significant impact on the fertility of bull semen and thus, on the reproductive performance of livestock. This research aimed to observe effect *thawing* methods on frozen semen characteristics of Simmental bull. This research was conducted at the Laboratory of Animal Physiology and Reproductive, Faculty of Animal Science, Universitas Gadjah Mada. Forty of frozen semen straws of Simmental bull were used in this research. The frozen semen was distributed into two *thawing* methods (28ºC for 30 seconds and 28ºC for 45 seconds). The parameters observed included motility of individual spermatozoa, viability, abnormalities and plasma membrane integrity of spermatozoa. The sperm smear was stained using the eosin-nigrosine, and plasma membrane integrity were observed using Hypoosmotic Swelling Test (HOS-Test). The data were analysed using Student's T-test. The result showed that *thawing* methods had a significant effect on motility and plasma membrane integrity ($P<0.05$). However, thawing methods had no significant effect on viability and abnormalities ($P>0.05$). The sperm motility and plasma membrane integrity from thawing methods 28ºC for 30 seconds and 28ºC for 45 seconds were 43.80±0.62%; 42.45±0.83%; and 65.12±1.42%; 67.02±1.33%, respectively. The sperm abnormality and plasma membrane integrity were 10.62±0.45%; 11.60± 0.45 and 76.47±0.69% and 73.80±1.08%. Based on the result, it could be concluded that thawing methods had effect on the motility and plasma membrane integrity. However, thawing methods had no effect on sperm viability and abnormality.

Keywords: Semen, Sperm, Plasma membrane Integrity, Simmental Cattle, Thawing

O-19 Efficacy of *Moringa oleifera* (MO) Extract in Treating Male Infertility Associated with Sperm DNA Fragmentation

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Comorbidities associated with diabetes mellitus and obesity are often linked to male infertility. These metabolic disorders induce sperm DNA damage and lead to infertility. Sperm DNA fragmentation is a valuable marker for male reproductive health. *Moringa oleifera* (MO) has long been used in traditional medicine and reported to have therapeutic properties. Therefore, the objective of this study is to investigate the effects of MO leaf extract on diabetes mellitus and obesity, leading to an improvement in male fertility parameters in induced rats. A total of 42 male Sprague-Dawley rats were divided into two main groups: the control group and the treatment group. The MO treatment groups received doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg MO leaf extract, respectively. All rats, except those in the normal control group, were induced to develop obesity through a high-fat diet (HFD) for four weeks, followed by streptozotocin induction. The treatment was administered orally for 14 consecutive weeks. After completing the treatment, all rats were sacrificed for further analysis. The results showed a significant improvement in sperm quality and a reduction in the sperm DNA fragmentation (SDF) index in the MO-treated group ($P < 0.05$) compared to all control groups. The Lee Index and blood glucose levels also demonstrated a significant improvement in the MO-treated group. In conclusion, this study provides evidence that MO leaf extract possesses anti-diabetic and anti-obesity properties, thus acting as a male fertility agent by improving sperm quality and reducing the SDF index.

Keywords: Infertility, Diabetes Mellitus, Obesity, *Moringa oleifera*, Sperm DNA Fragmentation

O-20 Different Response of *In Vivo* and *In Vitro* Fertilized Embryos Against Supplementation of Riboflavin and Pyridoxine During the Preimplantation Period

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Vitamin supplementation has been widely used for prenatal consumption to improve pregnancy quality. Riboflavin (B2) and pyridoxine (B6) vitamins contribute to one-carbon metabolism, which leads to epigenetic modifications via the metabolites generated. To establish the impacts on embryo quality, we explored varied reactions of direct addition of vitamins to *in vitro* and *in vivo* fertilized (IVF and IVV) and *in vitro* cultured embryos. Following superovulation in ICR female mice, *in vitro* fertilization and natural mating were used to create IVF and IVV embryos, respectively. From 2-cell stage until blastocysts, embryos were cultured in media of CZB (control), CZB with eliminated BSA (CZB-BSA), CZB with the addition of vitamins (CZB+B2B6), and CZB with the elimination of BSA and addition of vitamins (CZB-BSA+B2B6). We observed the developmental rates and cell lineages using Cdx2 and Nanog protein expression. Nucleolus morphological maturation was also monitored by NOPP140 immunostaining. As a result, vitamin addition had no effect on blastocyst development rates. The differences in cell lineages across fertilization environments were significant. IVV affected Nanog-expressed cells in CZB+B2B6, whereas IVF embryos affected Cdx2-expressed cells. Nucleolus compaction rates were greater in IVV embryos than in IVF but with vitamin supplementation, it was reduced. Thus, the addition of vitamins with BSA showed a positive response in IVF compared to IVV embryos during preimplantation period. Our findings suggest that embryonic regulatory systems change depending on the way of fertilization, resulting in a varied response to environmental exposures. Future studies will evaluate the consequences of regulatory changes on prospective illnesses.

Keywords: Embryo response, Riboflavin, Pyridoxine, *In Vitro* Fertilization, *In Vivo* Fertilization, Embryo Development, DoHAD

O-21 Unveiling the Impact of Vitamin D: Investigating Sirt1, Antioxidants, and Female Infertility

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Crosstalk between SIRT1 deficiency, Vitamin D (VD) insufficiency, and oxidative imbalance in the hypothalamic-pituitary-ovarian axis, may lead to compromised oocyte quality and infertility. Examining SIRT1's involvement in inflammation, mitochondrial function, and apoptosis highlights its significance for fertility. Reciprocal links between VD levels and SIRT1 activity are explored, alongside the ramifications of their scarcities, encompassing cell membrane instability, heightened autophagy, DNA damage, and elevated reactive oxygen species. Quantifying VD, SIRT1, antioxidants, and oxidants in infertility patients aims to untangle their complex interactions, shedding light on female infertility intricacies. Methods: This cross-sectional study included 342 (135 infertile and 207 fertile) female subjects. Serum levels of MnSOD, SIRT1, visfatin, GR, VD, adrenaline, and cortisol were analyzed by ELISA and were compared in fertile and infertile samples using the Mann-Whitney U test. Results: There were significantly high levels of VD, SIRT1, GR, MnSOD and visfatin in fertile female participants. However, mean adrenaline and cortisol levels were higher in infertile samples with a significant negative correlation with VD. A significant negative correlation of VD with MnSOD, SIRT1, visfatin and GR was observed ($p < 0.01$). In VD subset groups, MnSOD levels were significantly high in VD sufficient groups however, adrenaline and cortisol levels were significantly high in groups suffering from VD deficiency. Conclusion: Insufficient Vitamin D (VD) levels are linked to decreased SIRT1 activity and diminished antioxidants, potentially impeding natural reproductive processes and causing infertility. Further investigation is necessary to establish the cause-and-effect relationship between VD deficiency, conception, and the underlying mechanisms involved.

Keywords: Vitamin D, SIRT1, Oxidative Stress, Oxidants, Antioxidants, Female Infertility

O-22 Iron Dysregulation Promotes Ferroptosis in Allergic Asthma Pregnant Mouse Model

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Current evidence indicates iron-regulated cell death, termed ferroptosis in reproductive disorders. Although excessive iron intake and/or high iron status is implicated in endometriosis and preeclampsia, less is established about ferroptosis in allergic asthma during pregnancy. This study aimed to evaluate iron markers of ferritin, total iron binding capacity (TIBC) and heme oxygenase-1 (HMOX1) in allergic asthma pregnant mouse model. Twelve female ICR mice were divided into G1(control) and G2 (150μg/200μl ovalbumin (OVA)-induced allergic asthma). Animals in G2 were induced with allergic asthma through a series of OVA-sensitisation and -challenge, followed by methacholine (MCh) test. To induce follicular maturation and ovulation, animals were super ovulated and mated with fertile males. Following treatment, animals were euthanized. Serum and lung tissues were subjected to total iron binding capacity (TIBC), ferritin and heme oxygenase-1 (HMOX1) measurements using enzyme linked immune sorbent assay (ELISA). Both serum and lung HMOX1 were increased ($p<0.001$ and $p<0.001$). Serum TIBC was also upregulated ($p<0.001$). However, serum and lung ferritin were insignificant. Data suggests that OVA limits cell ability to bind to iron and promotes free/unbound iron leading to elevated TIBC. OVA also caused elevated pro-ferroptosis enzyme HMOX-1 leading to ferroptotic cell death. We conclude that OVA-induced allergic asthma promoted ferroptosis via the dysregulation of iron homeostasis. Elucidation into placental iron transfer and the regulation of specific T cell sub populations in this model, will establish iron metabolism in ferroptosis. Similarly, functional characterization of ferritin-TIBC-HMOX-1 autophagy pathway in in ferroptotic condition will provide insight into the treatment of ferroptopathy (elemental iron-associated diseases).

Keywords: Ovalbumin (OVA), Iron, Ferroptosis, Pregnancy, Ferritin, Total Iron Binding Capacity (TIBC), Heme oxygenase-1 (HMOX1)

O-23 *In-vitro* treatment with Vitamin D improves Human Sperm Motility and Protein Expression

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Vitamin D is a steroidogenic hormone that plays a crucial role in many physiological functions. Recently, researchers have been interested in exploring the relationship between vitamin D deficiency and male infertility, especially in serum samples and reproductive organs. However, the impact of in-vitro vitamin D treatment on ejaculated sperm remains unclear. The aim of this study is to investigate whether in-vitro vitamin D treatment can improve sperm motility, capacitation, and protein expression. We collected 40 semen samples from 20 fertile and 20 infertile men. Each sample was divided into two aliquots, one to serve as a control and the other to receive 1nM in-vitro vitamin D treatment for one hour. After treatment, we analyzed the sperm total motility, intracellular calcium release, and immunofluorescence of Phospholipase C Zeta (PLCζ) to compare the effectiveness of in-vitro vitamin D treatment in fertile and infertile semen samples. The results showed that in-vitro vitamin D treatment significantly improved the total motility of sperm in both fertile men (MD=4.65, p=0.0116) and infertile men (MD=4.25, p=0.0153). Furthermore, the treatment also significantly increased the intracellular calcium release in both groups (MD=4426, p=0.0004 for fertile men and MD=1315, p=0.0293 for infertile men). We also found that the fluorescence intensity of PLCζ was higher in the treatment group than in the control group for both fertile (MD=0.01408, p=0.0007) and infertile men (MD=0.001044, p=0.3237). In conclusion, our study suggests that in-vitro vitamin D treatment can enhance sperm quality by increasing intracellular calcium mobilization, which leads to improved sperm motility and protein expression. Therefore, we propose that vitamin D could be a useful tool in sperm preparation media.

Keywords: Vitamin D, Male Infertility, Capacitation, PLCζ, Intracellular Calcium.

O-24 Sub-acute and Sub chronic Exposure of Bisphenol F Induced Estrogen-like Effect in Male *Sprague-Dawley* Rats

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The utilisation of bisphenol A (BPA) has been banned due to its significant adverse impact on human health, characterised by its extensive toxicity. Therefore, bisphenol F (BPF) emerges as primary alternative. Because of rapid replacement of BPF, the toxicity studies and the safety level of BPF for human health are being question. Therefore, this study investigated the effects of BPF on male reproductive system of *Sprague-Dawley* rats by evaluating the testosterone, estradiol, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in plasma, oxidative stress status (malondialdehyde (MDA) and reduced glutathione (GSH) and histology observation of testis after subacute and sub-chronic exposures. Male *Sprague-Dawley* rats were administered with different dosage of BPF (1 mg/kg/bw, 5 mg/kg/bw, and 10 mg/kg/bw) via oral gavage for subacute (28 days; n= 20) and sub-chronic (60 days; n= 28). The results demonstrated that after subacute and sub-chronic exposure, estradiol significantly increased in BPF10 compared to the control ($p<0.05$). After sub-chronic exposure, testosterone levels in BPF10 were shown to be significantly lower than the control group ($p<0.05$). Although there were no significant differences in LH levels among experimental groups, an increased trend of FSH in a dose-dependent manner was observed. The MDA and GSH levels were found to be unchanged. The histological analysis of the testis showed the exfoliation and degeneration of seminiferous tubules, which suggested the presence of an estrogen-like effect. Hence, subacute and sub-chronic exposures to BPF induced estrogen-like effects in the seminiferous tubule of *Sprague-Dawley* rats leading to spermatogenesis defect without oxidative stress disturbance.

Keywords: Bisphenol F, EDCs, Estrogen-like Effect, Oxidative Stress, Testosterone, Estradiol, Testis.

O-25 Ferroptosis and Intracellular Senescence During Embryonic Development: A Systematic Review

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Ferroptosis is characterized by iron-dependent accumulation of lipid peroxides, which disrupts cellular membrane resulting in iron-toxicity cell death. Recent research links ferroptosis with the pathophysiological mechanisms of various diseases, including reproductive disorders. Ferroptosis may harm embryonic development by upsetting iron metabolism and cellular redox balance. Understanding the regulation of cell ferroptosis will establish its functional alterations, molecular mechanism and allow appropriate measures to prevent its onset as well as progression of such disorders. In this review, the possible effects of ferroptosis on embryo development and implantation are highlighted. We evaluated literatures related to ferroptosis and embryo growth. Databases (PubMed, WOS, and Scopus) showed one hundred and sixty-five studies based on "Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA)" in the screened publications until July 2023. Following language, article type, title, abstract, and accessibility of the full text, 21 studies were included in this review. Results showed several studies with positive ferroptosis effects in embryo development that were translated into poor blastocyst developmental capacities, reduced implantation potentials with risks to fetal outcome. The review clarifies complex intracellular communication in elucidating the precise roles and regulatory mechanisms of ferroptosis during embryogenesis. Chemical crosstalk between ferroptotic cell death and embryo developmental capacities that predicts successful embryo implantation warrants further investigation. Likewise, determining various factors including genetic predisposition will elucidate the susceptibility of embryonic cells in ferroptotic condition.

Keywords: Ferroptosis, Lipid Peroxidation, Cellular Redox Balance, Embryo Developmental Capacities, Implantation

O-26 Protective role of *Cocos nucifera* L. water on BPA-mediated oxidative stress and reproductive health damage in male rats

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Epidemiological studies have associated escalating bisphenol A (BPA) levels and a discernible rise in male infertility rates. *Cocos nucifera* L. (coconut) water is a diet rich with antioxidants and effective in inhibiting oxidative stress. The aim of the study is to evaluate the protective efficacy of coconut water on BPA-mediated oxidative stress in male rats. Thirty Sprague-Dawley rats were divided into control (C) received distilled water (0.5mL/day), vehicle (V) received corn oil (0.5mL/day), BPA (B) (50mg/kg/day), coconut water (CW) (10mL/kg/day) and coconut water plus BPA (CW-B) groups. The testis and epididymis were harvested on day 31 for oxidative stress markers levels, immunofluorescence assay, and sperm motility. BPA (B) group demonstrated a significant increase in malondialdehyde levels in the testis (275.86±5.34ng/mL) and epididymis (74.48±5.22ng/mL) while glutathione levels were significantly decreased in the testis (1.03±0.06µg/mL) and epididymis (0.23±0.02µg/mL) ($p<0.05$). Furthermore, grayscale intensity for actin (15.49±0.52) and tubulin (7.54±0.24) immunofluorescence of B group sperm were significantly reduced ($p<0.05$). The sperm motility in B group was also declined significantly (60.00±1.41%) ($p<0.05$). However, these parameters were normalized in CW-B groups. Collectively, reduced sperm motility affected by sperm cytoskeletal damage due to BPA was significantly improved by the protective role of coconut water in reducing lipid peroxidation and improving the antioxidant defense system. Present evidence indicates that coconut water possesses antioxidant-enhancing properties, escalating defense mechanisms, and is effective in managing male reproductive health in rats. Hence, suggesting *C. nucifera* L. water as a potential natural product for preventing BPA-induced male reproductive damage in rats.

Keywords: Coconut water, Bisphenol A, Testis, Oxidative stress, Antioxidant

O-27 Kelulut Honey Alleviates Ovarian Steroidogenic Enzymes Profiles in Letrozole-Induced Polycystic Ovary Syndrome Rats

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Polycystic ovary syndrome (PCOS) is an interrelated disorder of the reproductive, endocrine and metabolic systems. It is one of the most common disorders in women of reproductive age, affecting up to 20% of women worldwide. Currently, there is no definitive cure for PCOS. Therefore, treatment for PCOS usually focuses on disease management and depends on the symptoms. PCOS is treated with two medications: Metformin, which controls hyperglycaemia, and clomiphene citrate, which triggers ovulation. However, these drugs seem to be associated with various side effects. Therefore, it is of great interest to find a natural complementary therapy for PCOS that has fewer adverse effects. In animal studies, Kelulut honey (KH) has been shown to improve both female and male reproductive system abnormalities. Here we investigated the effects of isolated and combined KH, metformin and clomiphene on the improvement of steroidogenic enzyme profiles in letrozole-induced PCOS rats. Letrozole (1 mg/kg/day) was administered to female Sprague–Dawley (SD) rats for 21 days to induce PCOS. The PCOS rats were then divided into six experimental groups: untreated, treatment with metformin (500 mg/kg/day), clomiphene (2 mg/kg/day), KH (1 g/kg/day), combined KH (1 g/kg/day) and metformin (500 mg/kg/day) and combined KH (1 g/kg/day) and clomiphene (2 mg/kg/day). All treatments were administered orally for 35 days. We found that KH was comparable to clomiphene and metformin in improving the expression of Cyp17a1 and Cyp19a1. The efficacy of KH in restoring the altered profiles of steroidogenic enzymes in PCOS warrants a future clinical trial to clinically validate its therapeutic effect.

Keywords: Kelulut honey; Steroidogenic; Aromatase; PCOS

O-28 Bioactivity and Pharmaceutical Potential of *Phoenix dactylifera* on Morphine-Induced Toxicity in Male Rats: An Animal Model Study

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There are concerns over Malaysia's rising morphine use for treating terminally ill patients, which interferes with hormonal pathways. The purpose of this study was to assess the bioactive compounds of *P. dactylifera* and its potential effect on oxidative stress and testosterone of morphine treated male rats. Mature adult Sprague Dawley rat were randomly divided into: Group 1, force-fed with distilled water, 1 ml/kg BW for 35 days; Group 2, intramuscularly (i.m) injected with morphine, 20 mg/kg BW for 7 days; Group 3, force-fed with crude *P. dactylifera* extract, 200 mg/kg for 28 days; Group 4, injected (i.m) with morphine, 20 mg/kg BW for 7 days followed by force-fed of crude *P. dactylifera* extract, 200 mg/kg for 28 days. The rats were anesthetized prior to blood collection and then euthanized for testis extraction. The results of bioactive compounds analysis in *P. dactylifera* showed that there were presence of total phenolic compound (TPC) (151.04 mg/g) and saponin (10.2 mg/g). However, total flavonoids (TF) value was below method's threshold for detection. Supplementation of *P. dactylifera* in morphine treated rats affects the oxidative stress by significantly decreased malondialdehyde (MDA) (6.84 ± 0.65 ng/ml) and improved glutathione (GSH) (16.99 ± 0.24 ng/ml). Interestingly, there was also significant increase in testosterone levels after administration of *P. dactylifera* (8.93 ± 0.06 ng/ml). Therefore, present study indicates that bioactivity of *P. dactylifera* may directly affect the activation of endogenous testosterone synthesis and/or through oxidative stress reduction which gives a therapeutic benefit on morphine-induced male infertility.

Keywords: Phoenix dactylifera, Morphine-Induced Toxicity, Rats

O-29 Effects of Glutathione on Sperm Count and Sperm Morphology in STZ-Induced Diabetic Mice

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Oxidative stress in diabetes is known to lead to sperm defects and reproductive dysfunction. Glutathione (GSH) is an important antioxidant that protects sperm from oxidative stress and is essential for fertility and spermatogenesis. The aim of this study is to determine the effect of exogenous GSH on reproductive sperm quality in diabetic mice. Exogenous GSH could be a safe alternative to alleviate the effects of oxidative stress in diabetes mellitus and could be used clinically in combination with other known antidiabetic drugs. We investigated whether GSH could contribute to the recovery of spermatogenic dysfunction in STZ-induced diabetic BALB/c mice. Twelve BALB/c mice (6-8 w/o, n=6) were divided into two groups (i) diabetic control group and (ii) glutathione treated diabetic group. The treatment groups were injected 50 mg/kg body weight streptozotocin (STZ) intraperitoneally (i.p.) for 5 consecutive days to induce diabetes. The diabetic mice were administered 15 mg/kg body weight GSH i.p. weekly for 5 weeks. The diabetic mice in the control group showed significant deterioration in sperm parameters (sperm count, sperm motility, and sperm morphology) (*$p < 0.05$). Treatment with GSH restored sperm count, sperm motility, and sperm morphology and reduced sperm morphological abnormalities in diabetic mice. The mechanism is probably due to the antioxidant effect of GSH.

Keywords: Diabetes, Glutathione, Male infertility, Sperm count, Sperm Morphology

O-30 Protective effect of Stigmasterol against hydrogen peroxide-induced oxidative stress in mouse testes and human sperm: Experimental and computational approach

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Stigmasterol, a naturally occurring phytosterol, has been found useful to counteract oxidative stress in many tissues. Aim: To study the protective effect of stigmasterol against oxidative stress in the reproductive system of male mice and in human sperm. Methods: Oxidative stress was induced in male mice by administering 2% hydrogen peroxide (H_2O_2) orally for 7 days. Subsequently, Stigmasterol (SM) (10 and 20 mg/kg body weight) was given orally to these mice for another 7 days. At the end of the experiment, testes and epididymal sperm were harvested and sperm parameters, testicular histopathology and serum reproductive hormones were evaluated. Molecular biology techniques were used to evaluate the oxidative stress levels in mice's testes and sperm. Additionally, steroidogenesis and spermatogenesis in mice testes were also identified. Meanwhile, semen samples from fertile men were exposed to 200 µM H_2O_2 with SM (10 and 20 µg/ml) simultaneously, and were then evaluated for sperm parameters changes and antioxidant enzyme levels. Molecular docking study was also used to identify the binding affinity between several receptor proteins and SM. Results: In mice treated with H_2O_2, there was a reduction in sperm quality, alterations in testicular histology and reproductive hormones' levels, increased lipid peroxidation and reduced levels of antioxidant enzymes, steroidogenesis and spermatogenesis markers, while treatment with SM ameliorated these changes. SM improves the sperm parameters and increases the antioxidant enzyme levels in H_2O_2-exposed human sperm. Molecular docking analysis revealed that SM has a strong binding affinity with key protein receptors involved in oxidative stress, steroidogenesis and spermatogenesis. Conclusion: These findings suggest that SM could effectively safeguards male fertility by shielding the sperm and testes from oxidative damage caused by hydrogen peroxide.

Keywords: Stigmasterol, Oxidative stress, Mouse testes, Human Sperm

O-31 Effect of 35.5 GHz millimetre waves on reproductive system of male Wistar rat

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The increased usage and wide application of millimetre waves (MMW) in different spheres of public domain makes it important to have a better understanding of any possible health impacts caused by them. Mobile communication (5G bandwidth) and weather broadcasting radar works on millimetre waves. The aim of present study is to find out the effect of these radiation on reproductive system of male Wistar rat. Animals were divided into three groups Control, Sham-Exposed, and Exposed with 6 rats in each group. The experimental group were exposed to 35.5GHz frequency for 2 hrs/day for 60 days in a specially designed anechoic chamber. At the end of exposure various cauda epididymis and testis were excised out and various sperm parameters sperm count, viability, morphology, sperm mitochondrial activity was evaluated. Testis histopathological analysis was done by Johnson scoring. Ultrastructure analysis of sperm morphology was done by Scanning Electron Microscope (SEM). For oxidative stress analysis, Lipid peroxidation assay was done on sperm and testis homogenate. Results showed a significant decrease in sperm count and sperm viability in exposed group animals. SEM analysis showed changes in sperm head morphology. Histopathological changes also showed significant changes in seminiferous tubules and alteration in spermatogenesis in exposed group as compared to control. Lipid peroxidation also showed a significant increase in exposed group as compared to control. In conclusion results indicated that long term exposure to millimetre waves may affects male Infertility.

Keywords: Millimetre waves (MMW), Wistar rat, Sperm, Oxidative stress, Male infertility.

ABSTRACTS FOR POSTER PRESENTATION

P-1 Oxidative Stress Evaluation in Lineage-Related Toxicity of In-Utero Hydroquinone-exposed Hematopoeitic/Stem Progenator Cells of Maternal Mice

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Benzene is a known hematotoxic and leukemogenic agent and been reported to cause damage in multiple organs. Benzene through its metabolism exerts toxicity by producing reactive metabolite such as hydroquinone (HQ). Nevertheless, knowledge concerning oxidative stress-mediated mechanism of benzene-induced hematopoietic stem/progenitor cells (HSPCs) niche comprises of myeloid, lymphoid and erythroid lineages toxicity on maternal remains obscure. This study aimed to elucidate the effects of in utero HQ exposure on oxidative stress in lineage-related HSPCs of maternal mice. Briefly, pregnant mice (n=24) were divided into Control and HQ-treated groups. HQ was administered at 25 and 50 mg/kg body on gestational day (GD) 12, 14 and 16 followed by maternal bone marrow (BM) harvesting on GD18. Colony-forming Unit (CFU) assays were carried out to measure the clonogenicity status of myeloid, erythroid and pre-B lymphoid progenitors. Colonies-derived CFUs were counted and harvested for oxidative stress profile analysis. Results showed that HQ caused significant reduction ($p<0.05$) in colony counts for pre-B lymphoid progenitor at all HQ-exposed groups and erythroid progenitor at 50 mg/kg dosage. However, the colony counts for myeloid progenitors are not significantly affected. Meanwhile, HQ significantly decreased ($p<0.05$) glutathione (GSH) level only in myeloid progenitor while no effect was observed in superoxide dismutase (SOD) level for other cell types. Overall, HQ exposure significantly increased ($p<0.05$) lipid peroxidation in erythroid progenitor and showed no remarkable effect on protein oxidation for all cell types. In conclusion, HQ exposure has potential to induce oxidative damage in maternal HSPCs niche and the effect is dependent on hematopoietic cell lineages

Keywords: Cell lineage, Hematopoietic Stem/Progenitor cells, Hydroquinone, *In-utero*, Maternal, Oxidative stress

P-2 Bee Bread Ameliorates Steroidogenesis and Spermatogenesis Impairment in High-Fat Diet-Induced-Obesity Rat Model

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Steroidogenesis is essential for spermatogenesis; thus, any impairment in the process could lead to infertility. This study aimed to investigate the therapeutic effects of bee bread on steroidogenesis and spermatogenesis in obese male rats. Thirty-two adult male Sprague Dawley rats weighing 200-300 g were randomly assigned into four groups (n = 8/group), namely normal control (C), obese (Ob), Ob plus bee bread (Ob+BB), and Ob plus orlistat (Ob+OR) groups. Obesity was induced via a high-fat diet for 6 weeks. Bee bread (0.5 g/kg/day) or orlistat (10 mg/kg/day) dissolved in distilled water was given to the respective groups via oral gavage daily for 6 weeks following induction of obesity. The Ob group showed significant decreases in testicular mRNA levels of steroidogenic acute regulatory protein (StAR), cytochrome P450 enzyme (CYP11A1), CYP17A1, 3β-hydroxysteroid dehydrogenase (3β-HSD), and 17β-HSD enzymes compared to the control group. Meanwhile, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and testosterone hormone levels, sperm parameters [sperm count, viability, motility, and morphologically normal sperm] significantly decreased. In contrast, sperm nDNA fragmentation significantly increased in the Ob group compared to the control group. Treatment with bee bread revealed significant improvements in steroidogenic enzyme activities and its associated gene expression, reproductive hormone levels, sperm parameters, and a significant reduction in sperm nDNA fragmentation in obese male rats. Bee bread improved steroidogenesis and spermatogenesis by upregulating steroidogenic genes in obese rats. Therefore, bee bread exhibits promising therapeutic effects and may be regarded as a potential therapy to improve fertility in obese men.

Keywords: High-fat Diet, Obesity, Spermatogenesis, Steroidogenesis, Bee bread

P-3 Xylene Exposure during Early Gestation Impairs Feto-placental Dynamics and Induces Fetal Skeletal and Head Variations in Sprague-Dawley Rats

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This study aimed to investigate maternal and fetal toxicities from prenatal xylene exposure during the preimplantation period. Methodology: Twenty-four timed-pregnant Sprague Dawley rats (n = 6/group) were treated with 100, 500, and 1000 ppm of technical xylene including control during their preimplantation period (GD1 until GD3). On day 21 of gestation, the dams were subjected to caesarian hysterectomy for maternal and foetal data collection. Results: Study findings showed significant decrease in maternal body weight, corrected weight gain, and food intake in the 1000 ppm treated group during the treatment period compared to control. The data analysis showed highest percentage of preimplantation loss in the 500 and 1000 ppm-treated groups, even though it was not statistically significant. Placental tissue demonstrated a significantly higher percentage of glycogen cells in all xylene-treated animals, indicative of a restricted placental-fetal energy reserves transferred from the treated dams. Incidence of non-viable fetuses at term were observed in all xylene-treated groups. Analyses of the live fetuses revealed an increase in the prevalence of fetal skeletal and head variations in a concentration-dependent manner. Conclusion: The findings suggest that the adverse outcome of prenatal exposure to technical xylene was mediated by an impairment of feto-placental dynamic for normal fetal development. The dosage used in this animal study is lower compared to the current allowable limit of xylene exposure, when extrapolated into human dose. Therefore, we recommend to review the current safety measure against potential xylene hazards toward pregnant workers in order to safeguard maternal and fetal wellbeing.

Keywords: Xylene; Preimplantation; Fetal-skeletal; Feto-placental transfer; Microphthalmia; Anophthalmia; Gestation.

P-4 Oocyte Grading and Morphometry in the Indigenous Kedah-Kelantan Cattle Breed for Enhancement of Beef Cattle Genetics in Malaysia

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The aim of this study is to assess oocyte grading and morphometry in the Malaysian indigenous Kedah-Kelantan cattle breed, emphasizing their role in embryo quality during in vitro maturation. The embryo development is assessed up to the blastocyst stage, with a decreased in the development rate observed in the small-sized oocyte group. Therefore, a morphometry study is integral to categorize the oocytes according to their size. A total of 14 Kedah-Kelantan cattle ovaries were collected in a one-day program at the Bentong Slaughterhouse Centre in Pahang, swiftly transporting them to the laboratory within a two-hour timeframe at room temperature. The collected oocytes were classified into four categories (grade A, B, C, and D) based on cumulus cell layer morphology and cytoplasmic appearance. The oocytes and morphometric parameter were evaluated using an inverted microscope (Nikon, Japan) equipped with Bestscope software, BWHC2-4K Series 4K Digital Camera. The mean oocytes recovered were 44±0.62 with the median of 88. Out of the total 176 oocytes assessed, 54 oocytes (31%) were grade A, 34 oocytes (19%) as grade B, and an equal number of 44 oocytes (25%) falling into grade C and D categories. As in morphometric analysis, the mean oocytes diameter, perimeter and area were 13.20±0.99µm, 35.87±0.38µm. and 105.96±1.62µm2, $r$respectively. In conclusion, this study on oocyte grading and morphometry in Kedah-Kelantan cattle highlights the potential of using the local indigenous breed to advance the genetics of beef cattle in Malaysia through assisted reproductive technology.

Keywords: Assisted Reproductive Technology, *In-vitro* fertilization, Bovine, Production, Breed

P-5 Tocotrienol-Rich Fraction Modulates the Cell Cycle Signalling Pathway Genes in Preimplantation Embryos of Nicotine-Induced Oxidative Stress Model

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This study determined the effects of tocotrienol-rich fraction (TRF) on cell cycle signalling pathway related genes in preimplantation embryos subjected to maternal exposure of nicotine (Nic). Twenty-four female BALB/c mice were divided into four groups (G1-G4) and treated for 7 consecutive days: G1 [control group (0.9% NaCl)], G2 [Nic group (3mg/kg/day Nic)], G3 [Nic+TRF group (3 mg/kg/day Nic+60 mg/kg/day TRF)] and G4 [TRF group (60mg/kg/day TRF)]. Animals were superovulated before mating with fertile males. Plasma malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were evaluated using ELISA. Embryos with two and eight blastomeres were assessed for gene expression analysis using single-cell quantitative PCR (qPCR). Results showed that plasma MDA was markedly increased in G2 ($p<0.05$) with corresponding decrease in plasma GPx, CAT and SOD, compared to G1 ($p<0.05$). Concurrent treatment of Nic+TRF (G3) has normalized plasma MDA, GPx, CAT and SOD close to G1. At 2-cell stage, G2 showed the significant ($p<0.05$) upregulation of major cell cycle checkpoint regulators, including *Pten*, *Atm*, *p53*, *p21*, *p27* and Cyclin E/CDK2 complex. However, the expressions of *Pten, Atm, p53, p21*, *p27* genes and Cyclin E1/CDK2 complex were significant ($p<0.05$) downregulated in G3 and G4. The low presence of these genes was known to be important in the normal cell proliferation through the transition of G1/S phase of the cell cycle. Similar findings were noted at the 8-cell stage. This showed that TRF evidently has OS protection capacity and it could be through modulating the cell cycle signalling pathway.

Keywords: Tocotrienol-rich fraction, Nicotine, Oxidative stress, Preimplantation embryo, Cell cycle signalling pathway

P-6 Effects of Kombucha Extract Towards Metabolic Changes in Induced-Letrozole Polycystic Ovarian Syndrome (PCOS) in Rats: A Preliminary Study

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Polycystic ovary syndrome (PCOS) is a complex disorder that affects women of reproductive age and causes reproductive, endocrine, metabolic, and psychological health problems. It is characterised by high androgen levels, endometrial irregularities, and ovarian cysts. PCOS is associated with metabolic disorders, such as obesity, impaired glucose metabolism, insulin resistance, and dyslipidemia. Kombucha is produced by fermenting tea and sugar with a symbiotic culture of bacteria and yeasts (SCOBY) and has been suggested to have beneficial properties for treating PCOS symptoms. Thus, this preliminary study aimed to determine the effective dose of kombucha for metabolic changes in letrozole-induced PCOS rats. Letrozole was induced orally with 1 mg/kg/day for 28 days, and kombucha was orally administered with 200mg/kg/day, 400mg/kg/day, and 800mg/kg/day in 30 female Sprague-Dawley rats. Body Mass Index (BMI) showed no significant changes reduced in the kombucha treatment group. Polyphenols which are found in kombucha, known as catechins may have a slight impact on weight management. In the present study, a significant decrease in waist circumference was observed in 200mg/kg/day dosage as compared with the negative group ($p<0.001$). The polyphenolsin kombucha have potential effects to improve glucose resistance by regulating glucose homeostasis. This preliminary study demonstrated that kombucha, particularly at 200 mg/kg/day dosage has the potential to ameliorate the metabolism that occurs in PCOS.

Keywords: Kombucha extract, Polycystic Ovarian Syndrome, Physical activity, Blood glucose, Metabolic changes

P-7 Effect of Mature Coconut Water Supplement on the Reproductive Organ of BPA-Treated Female Rats

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Bisphenol A (BPA) mimics the effects of the hormone by attaching to estrogen receptors and obstructing hormone biosynthesis. Meanwhile, Cocos Nucifera L. (coconut) water full of nutrients and bioactive compounds was selected as a potential protective agent to counter the detrimental effects of BPA in the rat reproductive organ. In this study, the protective effect of Cocos Nucifera L. water on the reproductive organ of BPA-exposed rats was investigated. Twenty Sprague–Dawley rats were divided into five groups and force-fed with 0.5ml distilled water for DW group, 0.5ml corn oil for CO group, 50mg/kg of BPA for BPA group, 10mg/kg of coconut water for CW group and for CB group, 10mg/kg of coconut water and 50mg/kg of BPA. The rats were sacrificed on day 16. The ovary, oviduct and uterus were collected for histological examination. In general, the number of corpus luteum (3.00±0.32) and tertiary follicles (1.75±0.29) is higher in BPA group compared to DW, CO, CW and CB. BPA might stimulate the development and maturation of corpus luteum and tertiary follicles. Meanwhile, the width of myometrium 206.14±12.02 μm) and endometrium (403.53±23.96 μm) is higher in CW group compared to DW, CO, BPA and CB. This may be attributed to coconut water's potential estrogenic and antioxidant effects. However, the muscle thickness (65.63±3.24 μm) and the epithelial height of isthmus (16.89±0.85 μm) are higher in CO compared to BPA, DW, CW, and CB. This suggests that corn oil in CO and BPA might influence muscle thickness and epithelial height of isthmus.

Keywords: BPA, Coconut Water, Ovary, Oviduct, Uterus.

P-8 The Potential of Mature Coconut Water as a Dietary Supplement in Alleviating Oxidative Stress and Enhancing Sperm Quality

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Oxidative stress occurs when reactive oxygen species levels exceed the body's antioxidant defences, leading to decreased sperm quality in livestock animals. This adversely affects reproduction and causes losses in the livestock industry. Coconut (*Cocos nucifera L.*) water possesses antioxidant properties that reduce free radicals and increase antioxidant activity. However, mature coconut water (MCW) is perceived as an insignificant by-product due to limited knowledge of the impact of its administration to male fertility in ruminants. Therefore, this study aimed to explore the potential utilisation of MCW in improving Boer buck reproduction. Twelve sexually mature bucks were assigned to two groups, supplemented for 60 days with 5 mL/kg body weight of either plain water (Control) or mature coconut water (MCW) orally, with the same diet regime and water provided *ad libitum*. Collected semen was subjected to analyses for various sperm quality parameters. The oxidative stress markers, glutathione (GSH) and malondialdehyde (MDA) were measured using commercial ELISA kits. The MCW group showed notable increments ($p < 0.05$) in sperm motility (73.89 ± 0.26 %) and viability (87.16 ± 0.39 %), and a significant reduction ($p < 0.05$) in abnormal sperm morphologies (3.84 ± 0.09 %) compared to control bucks. ELISA assays for oxidative stress quantified significantly ($p < 0.05$) higher GSH level (4.12 ± 0.58 ng/mL) and lower MDA level (4.37 ± 0.52 ng/mL) within the MCW group compared to control. The findings highlight the potential of MCW in mitigating oxidative stress, likely attributable to its unique antioxidant constituents, such as cytokinins and L-arginine. Consequently, MCW could be a promising natural dietary supplement for enhancing male goat reproductive performance.

Keywords: Small Ruminant, Coconut water, Reproduction, Oxidative stress, Antioxidant

P-9 Palm Oil Tocotrienol Rich Fraction Ameliorate the Estrogen-Like Effects Induced by Bisphenol F in Testicular Tissue of Sprague Dawley Rats

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Bisphenol F (BPF) replaces BPA in manufacturing industry. BPF, like BPA, damages the male reproductive system through endocrine disruption. Tocotrineol Rich Fraction (TRF) from palm oil is a popular aphrodisiac agent and male reproductive system protector. TRF's effects on BPF-induced male reproductive system impairment are unknown. Therefore, this study examined the effects of TRF on male reproductive hormones, oxidative stress, and testicular morphology in BPF-induced Sprague Dawley rats. Adult male Sprague Dawley rats (n=40) were divided into five groups: control, TRF (EVNol SuprabioTM: 100 mg/kg), BPF (10 mg/kg), BE50 (BPF+TRF: 50 mg/kg), and BE100 (BPF+TRF: 100 mg/kg). All the substances were be given orally via force-feed needle for 28 days. Blood was drawn at the end of experiments to measure LH, T, and E2 levels and testes for oxidative damage and histological observation. TRF at the dose of 100 mg/kg enhanced the T levels and decreased E2 levels relative to the BPF group ($p<0.05$). Interestingly, no significant data were found on the oxidative stress status of the testicular tissue among groups. The testicular histopathology demonstrated TRF could reduce BPF's estrogen-like effects. In conclusion, TRF able to ameliorate the estrogen-like effects induced by BPF via its ability in controlling the testosterone levels without oxidative stress disturbance in male Sprague-Dawley rats.

Keywords: Endocrine, Testis, Vitamin E

P-10 The impact of preservation temperature and exposure time on Dorper ram Sperm Quality

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Artificial insemination (AI) in small ruminant with cryopreserved semen often results in low conception rates due to low post-thawed sperm survival. Alternatively, AI in small ruminant can be conducted using liquid-chilled semen due to higher survival rates. Present study was carried out to observe the quality of Dorper ram sperm in liquid-chilled (4°C) and cryopreservation (-196°C) for up to 96H. Semen ejaculates were collected from four sexually mature and healthy adult Dorper rams aged 2-3 years using an artificial vagina. The semen was centrifuged for 10min at 3000 x g and diluted with Tris-egg yolk extender. The semen-extender mixture was kept in a fridge at 4oC liquid-chilled for group 1, and in liquid nitrogen at -196oC for group 2, cryopreservation. Sperm acrosome integrity, membrane integrity, viability and morphometric properties were evaluated at every 24H up to 96H. In general, there were significant difference between liquid-chilled and cryopreservation for all sperm parameters ($p<0.05$). Significant differences were also observed in all sperm parameters for storage period, 24H to 96H ($p<0.05$). Percentage of membrane integrity (71.06±0.43%), acrosomal integrity (81.97±0.63%), viability (77.66±0.71%) and normal morphology (75.06±0.28%) of liquid-chilled sperm at 96H were significantly higher than the cryopreserved sperm. In conclusion, it is worth noting that cryopreserved sperm consistently exhibits diminished proportions of sperm physical and morphometric properties in comparison to liquid-chilled sperm. Storage period is also important to be considered when sperm needs to be preserved since it will affect the sperm parameters and thus reduce survivability and affect successful rate of AI.

Keywords: Ram Semen, Liquid-chilled, Cryopreservation, Dorper

P-11 The Effect of Exogenous Coenzyme Q10 Supplementation on Mitochondrial Intensity of Vitrified Mouse Embryo

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Mitochondria serve a vital role in embryo development by providing energy. However, vitrification often damages embryo's mitochondria, which further hinders embryo development. By preventing mitochondrial activity from getting damaged by vitrification, embryo development efficiency could thus be increased. Supplementation of coenzyme Q10 in culture media could overcome the mitochondrial dysfunction of embryos after vitrification. Objective: To investigate the effect of coenzyme Q10 supplementations in culture media on mitochondrial distribution and intensity of vitrified embryos. Method: Eight-cell stage embryos were vitrified using the EFS20/40 method. After thawing, embryos were divided into four groups (control non-vitrified, CoQ10 non-vitrified, control vitrified and CoQ10 vitrified). These groups were cultured for 2 hours in culture media. The embryos were stained after vitrification process by using MitoTracker Red dye for mitochondria and DAPI for nucleus of the embryos. Results were analyzed using an ANOVA test. Results: The results demonstrated that CoQ10 supplemented embryos had significantly higher mitochondrial intensities in both non-vitrified (67.02% $\pm$ 16.33 versus 55.37% $\pm$ 19.23) and vitrified groups (58.84 $\pm$ 9.23 versus 49.92 $\pm$ 17.74) compared to the control group ($p<0.05$). Conclusion: The supplementation of CoQ10 in the culture medium shows promise in enhancing mitochondrial intensities in vitrified mouse embryos. These findings suggest that CoQ10 supplementation could be an effective strategy to improve the success of Assisted Reproductive Technology (ART).

Keywords: Coenzyme Q10, Vitrification, EFS 20/40, Embryo, Mitochondria

P-12 Conventional Incubator vs. Time-lapsed Incubator Culture System: Comparison of Embryo Development and Subsequent Pregnancy Outcome

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Embryo incubation and assessment is the most crucial steps in IVF/ICSI programme. Therefore, providing a good culture and monitoring system ensures that the embryos are in optimal culture condition for growth and subsequently viable for implantation. Classically, embryos are taken out from a conventional incubator (CI) for daily assessment of development under a light microscope. Contemporarily, embryos are assessed in a time-lapse incubator (TLI) which programmed to record serial imaging of embryos in regular time intervals without removing the embryos from the incubator. In this retrospective study in our IVF unit, we aim to determine the benefit of the embryo incubation using TLI over the conventional incubation system and whether time-lapse system improves the pregnancy outcome. This study involved 342 patients undergoing IVF or ICSI with fresh embryo transfer cycles. Of these patients, 172 were culture in TLI and 170 were culture in CI. Clinical pregnancy rates in TLI group were significantly higher than in the CI group (43% vs. 24%; P = 0.0002). There were no statistical differences in fertilization and cleavage rates among these 2 groups. The proportion of blastocyst rates (45% vs. 26%; P < 0.0001) and frozen embryos (35% vs. 24%; P = 0.0036) are significantly higher in TLI group compared to CI group. Similar trend also noted in oocyte utilization rates (43% vs. 32%; P = 0.0057) and oocyte discard rates (57% vs. 68%; P = 0.0001). In a nutshell, time-lapse incubation and monitoring system have a significant benefit on clinical pregnancy rates, blastocyst rates and number of frozen embryos.

Keywords: Embryo Culture, Time-lapse, Incubator, Blastocyst, Pregnancy Rate

P-13 Fertilizing Capability of Low to Borderline Sperm Concentration in Conventional *In Vitro* Fertilization (IVF)

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Insemination via IVF depends on the capability of the sperm to be successful. Practically, it is performed in couples with normal sperm. According to the World Health Organization's (WHO) guidelines (6th edition), normal sperm parameters include sperm concentration higher than 16 million per ml, sperm motility greater than 42% and sperm morphology greater than 4%. Many studies have been conducted to predict the success of ART and its relationship with sperm parameters. However, it is still unclear whether using low to borderline sperm could result in oocytes fertilization. Couples who attended UMFertility reproductive centre from 2020 to 2022 with low to borderline sperm concentration, normal and acceptable sperm motility and morphology from fresh ejaculates were retrospectively included. Sperm concentrations of 10 to 23 million per millilitre were categorized as low to borderline. Insemination was performed according to the UMFertility standard operating procedure following guidelines from HFEA. The primary outcome was the percentage of oocytes fertilized. A total of 26 fresh IVF cycles were performed from 26 couples. The mean of sperm concentration was 18.08±3.99, mean for sperm motility 90.65±6.68 and mean for sperm morphology is 7.58±4.37. Univariate analyses showed that sperm parameters were not significant to be included in the final model. Sperm count (95% CI -1.041, 4.382; p=0.216), Sperm motility (95% CI -1.652, 1.705; p=0.974) and sperm morphology (95% CI -3.583, 1.447; p=0.389). According to our findings, low to borderline sperm concentrations can be used for insemination via IVF but must have good sperm motility and morphology. We suggest to use microdroplet culture to enhance fertilization.

Keywords: Male Infertility, *In-vitro* Fertilization, Assisted Reproduction, Sperm concentration, Sperm motility

P-14 Amelioration of Hydrogen peroxide (H_2O_2)-induced Oxidative stress in Testes and Sperm of mice by *Chlorophytum borivilianum* aqueous root extract

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H_2O_2 exposure can results in increased oxidative stress that can lead to the male reproductive dysfunction. Aims: To investigate the ability of *Chlorophytum borivilianum* (CB) aqueous root extract against H_2O_2-induced oxidative stress in testes and sperm of mice. Methods: Thirty adult ICR male mice were randomly divided into five (5) groups; (1) Normal control, (2) H_2O_2, (3) H_2O_2 + CB 100 mg/kg b.w., (4) H_2O_2 + CB 200 mg/kg b.w., and (5) H_2O_2 + Dimethyl fumarate 25 mg/kg b.w. (Positive control). Oxidative stress was induced by 2% H_2O_2 administered orally for seven days. Subsequently, CB was given orally at 10 and 20 mg/kg b.w. for another 7 days. At the end of the experimental period, testes and epididymal sperm were collected and sperm quality, testicular histology and serum reproductive hormones were examined. Molecular biological techniques were used to evaluate the oxidative stress levels in mice testes and sperm, as well as changes in steroidogenesis and spermatogenesis in mice testes. Results: Mice treated with H_2O_2 experienced a significant reduction in the sperm quality, alterations in testicular histology and reproductive hormones' level, increased lipid peroxidation, reduced antioxidant enzymes' levels and lowered steroidogenesis and spermatogenesis protein expression. Nevertheless, treatment with CB ameliorated these changes. Conclusion: These findings demonstrate that CB could help to protect the sperm and testes from oxidative stress-induced damage caused by hydrogen peroxide.

Keywords: Hydrogen Peroxide, *Chlorophytum borivilianum*, Oxidative stress, Testes, Sperm

P-15 Effect of Maternal Bisphenol A Exposure Below No Observed-Adverse-Effect-Level (NOAEL) on Apoptosis in BALB/c Mouse Embryos

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Bisphenol-A (BPA) is a widely used monomer in the manufacture of polycarbonate plastic. It is an endocrine disrupting chemical (EDC) that has the potential to interfere with the normal functions of the endocrine system, which can lead to reproductive disorders. In addition, BPA has the potential to alter apoptotic activity, as reflected in cytochrome C levels. Aim: This study aims to investigate apoptosis via the intensity of embryo cytochrome C release following BPA exposure below the no-observed-effect level (NOAEL) in adult female BALB/c mice. Methods: Mice were randomly assigned to either control Sham (Group 1), control Tween 80 + distilled water (Group 2), BPA treatment of 20 mg/kg body weight/day (Group 3), BPA treatment of 3 mg/kg body weight/day (Group 4) or BPA treatment of 1 mg/kg body weight/day (Group 5). The treatments were administered by oral gavage for 7 days. Mice were induced to super ovulate by intraperitoneal injection of Pregnant Mare Serum Gonadotrophin (PMSG) on the 4th day of treatment, followed by Human Chorionic Gonadotropin (HCG) 48 hours later. After completion of the 7-day treatment, the embryos were collected from the fallopian tube and fixed with 4 % paraformaldehyde. Cytochrome C immunofluorescence staining was then performed to measure the extent of apoptosis. Result: The results showed a significant increase in the release of cytochrome C in all groups treated with BPA. Conclusion: Maternal exposure to BPA below the NOAEL has the potential to cause an increase in cytochrome C intensity, indicating increased apoptosis in preimplantation embryos.

Keywords: Bisphenol A, Preimplantation Embryo, NOAEL, Reproduction, Immunofluorescent Staining

P-16 Comparison of Day-3 and Day-5 Embryo Transfer from Fresh and Frozen Cycles in determination of the IVF Pregnancy Outcomes

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Embryo transfer (ET) is a crucial step in assisted reproductive techniques (ART), commonly used in in-vitro fertilization (IVF). There are two types of ART cycles: fresh and frozen. To compare embryo quality and pregnancy rates between these two transfer methods, we conducted a retrospective analysis of laboratory data from January 2018 to December 2022. The study examined 697 patients who underwent IVF using either fresh ET cycles or frozen embryo transfer (FET) cycles. Of the 361 patients who underwent IVF with fresh ET cycles, 241 underwent Day 3 transfers and 120 underwent Day 5 transfers. Of the 336 patients who underwent FET, 119 underwent Day 3 transfers and 217 underwent Day 5 transfers. The study found that the quality of Day 3 and Day 5 embryos differed significantly in fresh cycles (P=0.002), but not significantly in frozen cycles (P=0.205). There was no significant difference in pregnancy rates for embryos transferred on Day 3 and Day 5 in fresh cycles (38.17% vs. 40%, P=0.150), but the difference was statistically significant in frozen cycles (32.77% vs. 47.47%, P=0.026). Implantation rates for embryos transferred on Day 3 and Day 5 in both fresh (31.54% vs. 35.83%, P=0.086) and frozen (26.05% vs. 41.47%, P=0.150) cycles were not statistically significant. Fresh and frozen embryo transfers on Day 3 and Day 5 are equally effective. There is no significant difference in embryo quality, pregnancy rates, and implantation rates between the two cycles, except for Day 5 embryo quality (P=0.004).

Keywords: Embryo Transfer, Frozen Embryo Transfer, Cleavage Stage, Blastocyst Stage, Embryo Quality

P-17 Effect of Dietary Supplementation of Mature Coconut Water on Semen Cryopreservation of Boer Bucks

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Cryopreservation is a method in livestock reproduction for preserving superior buck sperm for artificial insemination. Caprine sperm are more vulnerable to cryopreservation damage which exert extreme temperature variations, ice crystal formation, and stressors. Coconut water has been shown in studies to improve semen quality. Mature coconut water is an underutilized natural nutrient source. However, the effect of its dietary supplementation on the semen cryopreservation of Boer bucks is unknown. Hence to study this, twelve bucks were randomly assigned to group 1 (Control) and group 2 (MCW). Group 1 was treated with 5 ml/kg body weight of drinking water while group 2 was treated with 5 ml/kg body weight of mature coconut water for 30 days respectively. Semen samples were collected after 30 days and cryopreserved for 30 days. The post thaw semen quality was assessed. The result of this study showed significant improvements ($p<0.05$) to the semen total motility (55.63 ± 3.12%), progressive motility (42.16 ± 2.16 %), viability (67.90 ± 1.90%) and abnormal sperm morphology (5.60 ± 3.16%) compared to control (51.60 ± 3.18%), (37.61 ± 2.48%), (63.27 ± 1.44%), (7.40 ± 3.40%) respectively. The antioxidative properties of coconut water may have enhanced the antioxidant potential of the Boer bucks, thus reducing the damaging effects of reactive oxygen species during cryopreservation. The result highlighted mature coconut water potential as a dietary supplement to improve semen resilience against cryopreservation damage.

Keywords: Coconut Water, Reproduction, Cryopreservation, Small Ruminant

P-18 Characteristics and Quality of Life Among Menopausal Women in Banda Aceh, Indonesia

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The life expectancy of women in Indonesia including Aceh has increased significantly every year parallel with the improvement in accessing healthcare and medical advances. One third of women's life to be spent after menopause. Menopausal women will experience physical and psychological changes due to decreasing the production of female sex hormones which may impact on their quality of life. The aims of this study were to describe the characteristics and quality of life of menopausal women in Banda Aceh. Methods: This cross-sectional study was conducted at 9 health centers in each sub-district in Banda Aceh among 180 menopausal women. The Menopause-specific Quality of Life (MENQOL) Questionnaire was administered to assess their quality of life. The results reported that the most characteristics of the menopausal women in Banda Aceh had normal range of menarche age (98,9%), normal range of menopausal age (98,9%), menopause duration < 5 years (57,8%), upper secondary school level (48,3%), housewives (75%), married (61,7%), multiparity (59,4%), most had hypertension as comorbidity (27,9%), no history of gynecological surgery (88,5%) and no history of gynecological cancer (94,4%). Based on MENQOL Questionnaire showed most of the menopausal women had good quality of life (82,8%) with the most frequent symptoms on physical dimensions (58,15%) for those had menopause more than 5 years and vasomotor symptoms for those who had less. Conclusions: The results support that menopause causes both physical and psychiatric problems. Despite the menopausal women in Banda Aceh consider had good quality of Life, education, creating awareness and providing suitable intervention to improve their problem are still needed.

Keywords: Aceh, Characteristics, Menopause, MENQOL, Quality of Life

P-19 Ketapang Leaf (*Terminalia catappa L*) Extract Supplementation Increases the Expansion of Cumulus Cells in Beef Cattle Oocyte Maturation *In-Vitro*

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The aim of this research was to determine the effect of Ketapang (*Terminalia catappa L*.) leaf extract supplementation on increasing the expansion of cumulus cells in beef cattle oocyte maturation *medium in vitro.* This research was conducted from 20 May to 27 June at BET Cipelang Bogor. Data analysis used in this research was a Completely Randomized Design (CRD) with 4 P0 treatments as control, supplementation with Ketapang leaf extract at a dose of P1 7 mg/ml, P2 10 mg/ml, P3 13 mg/ml and 5 repetitions. Variables in this study include the success rate of oocyte maturation in *vitro*, characterized by expansion *of the cumulus oophorus*. The procedures used include Ketapang leaf extraction, ovary aspiration, oocyte selection, oocyte washing and *in vitro* oocyte maturation. The results of this study indicate that Supplementation of Ketapang Leaf Extract (*Terminalia catappa L*) has an effect ($P<0.05$) on oocyte cumulus expansion at a dose of 10 mg/ml P2 and 13 mg/ml P3 for *in vitro* maturation and for further research it is recommended to be carried out by increasing the amount dose variations to determine toxicity and efficacy.

Keywords: Ketapang Leaves, Oocytes, Cumulus Cells, Maturation

P-20 Complete Androgen Insensitivity Syndrome (CAIS): A Rare Case Report

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Complete Androgen Insensitivity Syndrome (CAIS) is rare sexual developmental disorders which can be easily misdiagnosed that affects 2 to 5 cases out of every 100.000 genetically male people as result of profound resistance of the androgen receptor towards the action of androgen. CAIS existed as a female which is characterized by having a chromosome karyotype of 46 XY with normal breast, no uterus, ovaries, and fallopian tube with a blind-ending vagina. In addition, there was the presence of bilateral undescended testis either in the inguinal canal, abdomen, or labioscrotal junction and elevated testosterone levels. Case: a 29-year-old patient who presented phenotypically as female and lived as a female presented with primary amenorrhea with full mammary gland on both sides with nipple dysplasia, female vulva with no axillary and pubic hair with a short blind-ending vagina. Ultrasound revealed uterus was difficult to be identified, the right ovary measuring 1,77 x 1,68 x 2,24 cm, and the left ovary measuring 1,33 x 1,16 x 1,61 cm. Laparoscopy examination revealed no uterus with normal ovaries. Her Karyotype analysis revealed 46 XY. Conclusion: Multidisciplinary approach that includes surgical excision of bilateral gonads to prevent malignancy, estrogen supplementation, and vaginoplasty along with thorough and repetitive psychological counseling remain the mainstay of treatment of CAIS.

Keywords: Complete Androgen Insensitivity Syndrome, Primary Amenorrhea, Gonads